

P<sub>n</sub> 6118

ISSN 0753-4973

# ALYTES

INTERNATIONAL JOURNAL OF BATRACHOLOGY



8 JAN 2003

December 2002

Volume 20, N° 1-2



**International Society for the Study  
and Conservation of Amphibians**  
(International Society of Batrachology)

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# ALYTES

INTERNATIONAL JOURNAL OF BATRACHOLOGY

December 2002

Volume 20, N° 1-2

*Alytes*, 2002, 20 (1-2): 1-12.

## The tadpole of *Ptychadena aequiplicata* (Werner, 1898) with the description of a new reproductive mode for the genus (Amphibia, Anura, Ranidae)

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We describe the tadpole of *Ptychadena aequiplicata* (Werner, 1898) based on specimens from Taï National Park, Ivory Coast. Compared to other tadpoles of the genus it is unique by its bicoloured body, posterior part lighter than anterior one. *Ptychadena aequiplicata* is restricted to closed forest habitats. It has a reproductive behaviour unique to the genus. Clutches of numerous females were communally deposited on the forest floor, between leaves and small plants. Spawning sites were always situated at the edges of dried up ponds. Pre-hatching time was variable. Tadpoles still hatched two weeks after oviposition. In some specimens development continued in the large eggs up to Gosner stage 28. Developmental time from hatching to metamorphosis was less than two weeks. We regard this developmental mode as an adaptation to the surprisingly high desiccation risks of the forest ponds in Taï National Park.

### INTRODUCTION

*Ptychadena aequiplicata* (Werner, 1898) is widespread in West and Central Africa (tab. 1). In its whole range it inhabits exclusively forest habitats (GUIBÉ & LAMOTTE, 1958; LAMOTTE, 1966; AMIET, 1974, 1975; LARGEN & DOWSETT-LEMAIRE, 1991), mainly primary rain forest (RIVA, 1994; BÖHME, 1994). While tadpole descriptions (GUIBÉ & LAMOTTE, 1958; LAMOTTE & ZUBER-VOGELI, 1953; LAMOTTE et al., 1958, 1959; LAMOTTE & PERRET, 1961; RÖDEL, 2000a) and biological data (BARBAULT & TREFAUT RODRIGUES, 1978; RÖDEL, 2000a) of many other



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Table 1. – Known distribution of *Ptychadena aequiplicata*.

Country	Source
Guinea	BÖHME, 1994
Liberia	GUIBÉ & LAMOTTE, 1957, 1958; SCHIÖTZ, 1968; EUZET et al., 1969
Ivory Coast	EUZET et al., 1969; LAMOTTE, 1967; RÖDEL, 2000b; this paper
Ghana	SCHIÖTZ, 1964a
Nigeria	SCHIÖTZ, 1963
Cameroon	PERRET, 1966; AMIET, 1974, 1975; LAWSON, 1993; FRETEY & BLANC, 2000
Central African Republic	FRETEY & BLANC, 2000
Republic of Congo	FRETEY & BLANC, 2000
Congo	LARGEN & DOWSETT-LEMAIRE, 1991; FRETEY & BLANC, 2000
Equatorial Guinea	RIVA, 1994; FRETEY & BLANC, 2000
Gaboon	FRETEY & BLANC, 2000

West African *Ptychadena* species are available, nearly nothing is known about the biology of this common forest frog. Its voice was made known by SCHIÖTZ (1964a) and AMIET (1974). In all *Ptychadena* species where reproduction is known, spawn is deposited as a surface layer in stagnant waters of variable size (WAGER, 1986; RÖDEL, 2000a). In Taï National Park (TNP), Ivory Coast, we were able to identify *P. aequiplicata* tadpoles in 1999. Those tadpoles have been collected in forest ponds and raised to metamorphosis to assure species affiliation. However, other details of the frog's reproductive biology, especially spawning sites and clutches, remained unknown. In 2000 we found *P. aequiplicata* clutches deposited terrestrially at the edges of dried up forest ponds.

## MATERIALS AND METHODS

### STUDY AREA AND FIELD DATA

The TNP is the largest protected area of rain forest in West Africa. Our main investigation area was located 23 km southeast of the small town of Taï and comprises about 30 km<sup>2</sup> of primary and secondary rain forest around the Station de Recherche en Ecologie Tropicale (SRET; 5°50'N, 7°20'W). Between 1991 and 1999, mean annual precipitation at the SRET was 1,854 mm (*sd* 249; range 1,424–2,194 mm; R. Noë, pers. comm.). Most precipitation occurred from April to July and from September to November. The first dry period lasted from December to February, normally a second one occurred in August. The mean annual temperature was about 25°C. More detailed descriptions of the TNP are provided by GUILLAUMET (1967) and RIEZEBOS et al. (1994).

Data were collected irregularly in different parts of the forest, and regularly along 10 transects, 600 m in length. Six transects have been set up in primary and four in secondary forest. Data collection and transect conception were described in more detail in RÖDEL (2000b) and RÖDEL et al. (in press a).

#### PRESERVATION AND DESCRIPTIVE METHODS

Frogs were sacrificed in a chlorbutole solution and preserved in 4 % formaldehyde or 70 % ethanol. Later on all adults were transferred into ethanol. Larvae of different stages were preserved in 4 % formaldehyde. Measurements were taken with a dial calliper ( $\pm 0.1$  mm) or a measuring ocular in a dissecting microscope ( $\pm 0.1$  mm; Zeiss Stemi SV 6). We measured body length (BL), body width (BW, measured at the plane of the eyes), tail length (TL), fin height (TF), height of tail axis (TA) and body height (BH). Measures are given in mean values with standard deviation. Nomenclature of morphological features follows VAN DIJK (1966), ALTIG & JOHNSTON (1989) and ALTIG & MCDIARMID (1999). The labial tooth row formula is according to DUBOIS (1995). Staging of tadpoles was according to GOSNER (1960). The tadpole description is a summary of all specimens from Gosner stages 27-38. The description of the coloration is based on living tadpoles. Drawings were done with the aid of a camera lucida. Voucher specimens are deposited in the collection of the Staatliches Museum für Naturkunde Stuttgart (SMNS 9774.1-6, 2 males, 4 females; 9775.1-15, tadpoles; 9776.1-73, tadpoles).

#### REARING

Tadpoles were reared in plastic aquaria (PT2 Firma Hoch, 25 × 15 cm, 16 cm water depth), filled with rain water and fed ad libitum with commercial fish food (TetraMin®). Water was changed every day. As development in nature seems to be much faster (see below), we don't give any developmental time tables. Species affiliation was assured by tadpoles captured in forest ponds and partly raised to metamorphosis in 1999 (SMNS 9775.1-15).

## RESULTS

#### TADPOLE DESCRIPTION

Body ovoid in dorsal view (fig. 1b); in lateral view slightly pointed (fig. 1a); body length  $1.70 \pm 0.08$  ( $n = 51$ ) times body width (measured at the plane of the eyes); body length  $0.61 \pm 0.05$  ( $n = 51$ ) tail length; eyes laterally; nostrils dorsolaterally, closer to snout tip than to anterior corner of the eyes; tail straight, if extrapolated, axis of tail passing through eyes; fin height nearly equal to body height ( $0.80 \pm 0.08$ ,  $n = 51$ ); dorsal fin originating anterior to tail-body junction; dorsal and ventral fin nearly parallel to tail axis; tail tip rounded; small oral disc anteroventral, bordered by two to three rows of papillae with large rostral gap, caudal with few larger papillae; many additional papillae grouped in oral angles; jaw sheaths massive and serrated; upper jaw sheath evenly broad U-shaped; lower jaw sheath V-shaped; labial tooth row formula of tadpoles, older than stage 28, 1:2+2/2 or 1:2+2/1+1:1 (fig. 2; formulae of all stages are summarized in tab. 2); spiracle sinistral, visible dorsally;

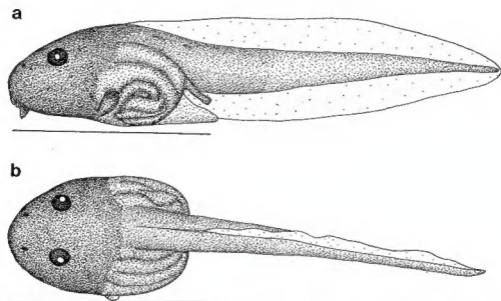


Fig. 1. – Lateral (a) and dorsal (b) view of a *Ptychadena aequiplicata* tadpole (stage 33) from Tai National Park, Ivory Coast. Scale bar: 10 mm.

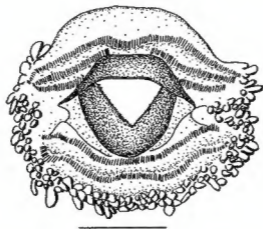


Fig. 2. – Oral disc of the specimen illustrated in fig. 1. Scale bar: 1 mm.

Table 2. – Labial tooth row formulae of *Ptychadena aequiplicata* tadpoles, sample size in parentheses. Stage after GOSNER (1960).

Stage	Labial tooth row formulae	Stage	Labial tooth row formulae
20	Not present (1)	31	1:2+2/1+1:1 (3); 1:2+2/2 (3)
22	Not present (1)	32	1:2+2/1+1:1 (4)
24	1/1+1:1 (1); 1:1+1/1+1:1 (4)	33	1:2+2/2 (2)
25	1/1+1:1 (6); 1:1+1/1+1:1 (3)	34	1:2+2/1+1:1 (3)
27	1:1+1/2 (1); 1:2+2/2 (1)	35	1:2+2/1+1:1 (1)
28	1:1+1/2 (4); 1:1+1/1+1:1 (3); 1:2+2/1+1:1 (3); 1:2+2/2 (6)	36	1:2+2/1+1:1 (6)
29	1:2+2/2 (3)	37	1:2+2/1+1:1 (7)
		38	1:2+2/1+1:1 (1)

vent opening medially, positioned basicaudally. Further measurements are summarized in tab. 3.

In life the tadpoles are bicoloured. The anterior part of the body is dark beige to brown, the posterior part is light brown to yellow. The tail axis becomes lighter towards the tail tip. The fin is transparent. The venter is slightly lighter than the back.

## BIOLOGY

In *P. aequiplicata* sexes differ considerably in size. Mean size in females was 48.3 mm (range 39-64,  $n = 8$ ), in males mean SVL was 39.5 mm (range 36-43,  $n = 6$ ). Two dissected females (SMNS 9774.5-6; 58 and 54 mm SVL) bore 457 and 478 ovarian eggs, respectively. The eggs had a diameter of about one mm, and black and white poles.

Male's choruses were regularly heard after heavy rainfall throughout the whole rainy season. Calling males were always concealed between leaves or roots, several meters apart from filled or dried up forest ponds. Calling activity was mainly during night, rarely during day. Adults were registered in swampy areas, as well as in relatively dry parts of the forest without open water. Compared to other leaf-litter frogs, *P. aequiplicata* was not very abundant. We found 0.15 *P. aequiplicata* per transect hour in primary forest (289 h). During 93 transect hours in secondary forest we found only one *P. aequiplicata*. Tadpoles were continuously encountered throughout the rainy season. We found them only in stagnant forest ponds within primary rain forest. Juveniles were registered between end of June and beginning of November. They measured  $14.8 \pm 2.8$  mm SVL (11-20 mm,  $n = 31$ ).

At 2 p.m. on September 8<sup>th</sup>, we heard a large chorus at a dry pond after rainfall (site 1). While approaching the spot, about 50 *P. aequiplicata* jumped away (J. Fahr, pers. comm.). On September 15<sup>th</sup>, we discovered more than 2000 eggs at this site. The eggs were deposited on the ground between the stalks of herbs. The eggs were congregated on two areas, measuring 1.5 m<sup>2</sup> and 2 m<sup>2</sup>, respectively. On September 18<sup>th</sup>, we found similar spawning sites at four other

Table 3. - *Ptychadena aequiplicata* tadpole measurements (mean  $\pm$  standard deviation). Only tadpoles for which all measurements were available were taken into consideration. For abbreviations see *Materials and methods*. Stage after GOSNER (1960).

Stage	n	BL	BH	BW	TL	TF	TA	BL+TL	BL/BW	BL/TL	BH/TF
20	1	2.6	1.3	1.3	3.6	1.4	0.5	6.2	2.0	0.7	0.9
22	2	2.6 $\pm$ 0.1	1.2 $\pm$ 0.1	1.3 $\pm$ 0	3.6 $\pm$ 0.2	1.4 $\pm$ 0.1	0.5 $\pm$ 0	6.1 $\pm$ 0.3	2.0 $\pm$ 0.1	0.7 $\pm$ 0	0.9 $\pm$ 0
24	5	3.4 $\pm$ 0.3	1.5 $\pm$ 0.2	2.0 $\pm$ 0.3	5.6 $\pm$ 1	1.8 $\pm$ 0.3	0.8 $\pm$ 0.3	9.0 $\pm$ 1.3	1.7 $\pm$ 0.1	0.6 $\pm$ 0.1	0.9 $\pm$ 0.2
25	9	3.2 $\pm$ 0.2	1.5 $\pm$ 0.2	1.9 $\pm$ 0.2	5.2 $\pm$ 0.4	1.7 $\pm$ 0.3	0.8 $\pm$ 0.4	8.4 $\pm$ 0.6	1.7 $\pm$ 0.1	0.6 $\pm$ 0	0.9 $\pm$ 0.1
27	2	4.3 $\pm$ 0.6	1.9 $\pm$ 0.3	2.7 $\pm$ 0.5	5.0 $\pm$ 3.0	2.3 $\pm$ 0.3	1.0 $\pm$ 0.1	9.3 $\pm$ 2.8	1.6 $\pm$ 0.1	1.4 $\pm$ 1.3	0.8 $\pm$ 0
28	16	5.0 $\pm$ 0.9	2.1 $\pm$ 0.3	3.1 $\pm$ 0.6	8.0 $\pm$ 1.2	2.8 $\pm$ 0.6	1.1 $\pm$ 0.1	13.0 $\pm$ 2.0	1.7 $\pm$ 0.1	0.6 $\pm$ 0.1	0.8 $\pm$ 0.1
29	3	6.5 $\pm$ 0.2	3.0 $\pm$ 0.2	4.0 $\pm$ 0.2	10.3 $\pm$ 0.3	3.7 $\pm$ 0.2	1.3 $\pm$ 0.1	16.9 $\pm$ 0.3	1.6 $\pm$ 0	0.6 $\pm$ 0	0.8 $\pm$ 0.1
31	6	8.8 $\pm$ 1.0	3.7 $\pm$ 0.7	5.3 $\pm$ 0.7	14.9 $\pm$ 2.0	4.9 $\pm$ 0.8	1.9 $\pm$ 0.3	23.7 $\pm$ 2.9	1.6 $\pm$ 0.1	0.6 $\pm$ 0	0.8 $\pm$ 0.1
32	4	8.9 $\pm$ 0.8	3.7 $\pm$ 0.1	5.3 $\pm$ 0.2	14.3 $\pm$ 0.8	4.7 $\pm$ 0.3	2.0 $\pm$ 0.1	23.2 $\pm$ 1.6	1.7 $\pm$ 0.1	0.6 $\pm$ 0	0.8 $\pm$ 0
33	2	9.2 $\pm$ 0.1	3.9 $\pm$ 0.1	5.5 $\pm$ 0.1	15.7 $\pm$ 0.6	4.7 $\pm$ 0	2.1 $\pm$ 0.1	24.8 $\pm$ 0.5	1.7 $\pm$ 0	0.6 $\pm$ 0	0.8 $\pm$ 0
34	3	9.2 $\pm$ 0.5	3.8 $\pm$ 0.3	5.7 $\pm$ 0.3	14.7 $\pm$ 0.9	4.7 $\pm$ 0.7	2.1 $\pm$ 0.1	23.9 $\pm$ 0.7	1.6 $\pm$ 0	0.6 $\pm$ 0.1	0.8 $\pm$ 0.1
35	1	9.3	4.1	5.4	15.0	4.9	2.9	24.3	1.7	0.6	0.8
36	6	9.9 $\pm$ 0.4	4.0 $\pm$ 0.3	5.8 $\pm$ 0.4	16.7 $\pm$ 1.1	5.1 $\pm$ 0.4	2.2 $\pm$ 0.2	26.6 $\pm$ 1.5	1.7 $\pm$ 0.1	0.6 $\pm$ 0	0.8 $\pm$ 0.1
37	7	10.1 $\pm$ 0.5	4.3 $\pm$ 0.3	6.2 $\pm$ 0.3	17.2 $\pm$ 1.7	4.9 $\pm$ 0.5	2.2 $\pm$ 0.2	27.2 $\pm$ 2.1	1.6 $\pm$ 0.1	0.6 $\pm$ 0	0.9 $\pm$ 0.1
38	1	11.0	4.9	6.3	17.0	5.2	2.2	28.0	1.7	0.6	0.9

forest ponds. These sites comprised several hundreds to thousand eggs, spread between leaf litter, restricted to a few square meters at the very border of the ponds. All five ponds were dried up at that time. Egg size was between 3.5 and 6 mm. At all but one site, all eggs were of the same stage. Four of the five ponds remained water filled only in the high rainy season between August and November (Rödel, unpubl.). All spawning sites were located such that eggs only came in contact to open water after the ponds reached their maximum possible water capacity.

We assume that the eggs (4-5 mm) we discovered at site 1 were deposited on or shortly after September 8<sup>th</sup>. On September 16<sup>th</sup>, we flooded 100 eggs of these eggs with water. At that time the original pond was also water-filled. Within 5 min, 95 tadpoles, stage 25, hatched. None of these tadpoles possessed external gills, but all still had large yolk sacks. However, they immediately started feeding on the provided fish food. The remaining five tadpoles were still alive but apparently not able to hatch. We regularly preserved tadpoles of this series (SMNS 9776.1-73). On September 25<sup>th</sup>, the tadpoles reached stages 37-38 and had a body length of 10-11 mm. At that time, *P. aequiplicata* tadpoles in the forest pond had nearly twice the size of the ad libitum fed captive ones, and were about to metamorphose. In nature, time from hatching to metamorphosis seemed to last about two weeks. We kept another 106 eggs of the same site until September 23<sup>rd</sup>. Development of these eggs continued until stage 28 (fig. 3). The diameter of the eggs increased slightly during development (tab. 4). After flooding the 106 eggs with water, 38 larvae (stage 28) hatched within 40 minutes. After 90 minutes, only six tadpoles remained within the jelly capsules, obviously not able to hatch. Hatching success therewith equalled those eggs that were flooded a week before. Presuming that these eggs were deposited the 8<sup>th</sup> or shortly after that date, they survived within eggs for two weeks.



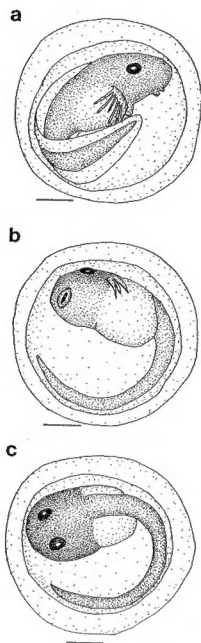


Fig. 3. – Lateral (a), ventral (b) and dorsal (c) view of egg stages of *Ptychadena aequiplicata*; (a) and (b) show individuals of stage 23, (c) figures a stage 25 tadpole. Scale bar: 1 mm.

Table 4. – Stage of pre-hatching *Ptychadena aequiplicata* and respective egg sizes.

Stage	n	Egg diameter	Stage	n	Egg diameter
20	2	4-4.7	24	2	4.8
21	8	4.2-5.1	25	2	5.5-5.7
22	3	4.4-5.1	27	2	5
23	2	5-5.3			

On September 18<sup>th</sup>, we collected much smaller eggs (3.5 mm) from a second site. We flooded 28 of these a day later. After 8 hours, only three tadpoles had hatched, all with external gills and large yolk sacks. After 24 hours, 13 tadpoles remained in the eggs, still alive. During the next 8 hours only four others managed to hatch.

We were able to monitor eggs at one site in the forest for one week. During that time it seemed that almost none of the eggs had disappeared, despite the fact that numerous ants, including driver ants (*Dorylus* sp.), were regularly encountered at that spot. We never detected any *P. aequiplicata* eggs that were covered with fungi.

## DISCUSSION

With the exception of its habitat preferences (e.g., AMIET, 1975; LARGEN & DOWSETT-LEMAIRE, 1991), its voice (SCHIÖTZ, 1964b; AMIET, 1974) and the size dimorphism in male and female *P. aequiplicata* (PERRET, 1966), nearly nothing was known about the biology of this widespread West and Central African forest frog. *Ptychadena aequiplicata* tadpoles differ from other West African *Ptychadena* larvae by their bicoloured body, anterior part darker than posterior one. Other species have a more or less uniform beige or brown body (LAMOTTE & ZUBER-VOGELI, 1953; GUIBÉ & LAMOTTE, 1958; LAMOTTE et al., 1958, 1959; LAMOTTE & PERRET, 1961; RÖDEL, 2000a). Another distinctive feature was the presence of three labial tooth rows in the upper lip in tadpoles more advanced than stage 28. Only a few individuals of *P. oxyrhynchus* and *P. mascareniensis* tadpoles are also known to possess three tooth rows in the upper lip (tab. 5).

While the free-swimming tadpoles fit the general characterisation of tadpoles of that genus (ALTIG & McDIARMID, 1999), being exotroph, lentic and benthic, clutch deposition clearly does not. All other known *Ptychadena* species deposit their eggs in a single layer as a surface film (WAGER, 1986; RÖDEL, 2000a). *P. aequiplicata* deposit its eggs on the forest floor at the border of dried up forest ponds. These eggs are larger than other *Ptychadena* eggs (compare with RÖDEL, 2000a). Consequently, *P. aequiplicata* females seem to produce smaller clutches (tab. 6).

Our experiments showed that *P. aequiplicata* is very variable in respect to hatching time, lasting from a few days to more than two weeks. This strategy is very similar to that of a

Table 5. – Labial tooth row formulae (LTRF) of *Ptychadena* tadpoles.

LTRF	<i>Ptychadena</i> species (source)
1/1+1:1	<i>schubotzi</i> (RÖDEL, 2000a)
1/2	<i>schubotzi</i> (RÖDEL, 2000a); <i>bibroni</i> (GUIBÉ & LAMOTTE, 1958; LAMOTTE & PERRET, 1961); <i>submascareniensis</i> (LAMOTTE et al., 1958); <i>trinodis</i> (LAMOTTE et al., 1958); <i>tourneri</i> (LAMOTTE et al., 1958; RÖDEL, 2000a)
1/2+2	<i>schubotzi</i> (RÖDEL, 2000a)
1:1+1/1	<i>bibroni</i> (RÖDEL, 2000a); <i>submascareniensis</i> (LAMOTTE et al., 1958)
1:1+1/2	<i>pumilio</i> (LAMOTTE et al., 1959); <i>longirostris</i> (RÖDEL, 2000a); <i>oxyrhynchus</i> (GUIBÉ & LAMOTTE, 1958; WAGER, 1986; LAMBIRIS, 1988; RÖDEL, 2000a); <i>trinodis</i> (LAMOTTE et al., 1958; RÖDEL, 2000a); <i>mascareniensis</i> (PERRET, 1966)
2/2	<i>oxyrhynchus</i> (LAMBIRIS, 1988, 1989)
1:1+1/3	<i>oxyrhynchus</i> (LAMOTTE & ZUBER-VOGELI, 1953)
1:2+2/2	<i>oxyrhynchus</i> (GUIBÉ & LAMOTTE, 1958); <i>mascareniensis</i> (PERRET, 1966)

Table 6. – Egg numbers of West African *Ptychadena* species.

Species	Egg numbers	Source
<i>P. aequuplicata</i>	457–478	This paper
<i>P. bibroni</i>	800–1500	RÖDEL, 2000a
<i>P. bibroni</i>	1333 ± 643	BARBAULT & TREFAUT RODRIGUEZ, 1978; BARBAULT, 1984
<i>P. schubotzi</i>	500–1000	RÖDEL, 2000a
<i>P. schubotzi</i>	2011 ± 851	BARBAULT, 1984
<i>P. oxyrhynchus</i>	3476 ± 1542	BARBAULT & TREFAUT RODRIGUEZ, 1978; BARBAULT, 1984
<i>P. mascareniensis</i>	1079	BARBAULT, 1984

savannah frog, *Hemisus marmoratus*, who has to cope with rather unpredictable environmental conditions (RÖDEL et al., 1995; KAMINSKY et al., 1999). Forest ponds in TNP dry up surprisingly often, even during the rainy season (Rodel, unpubl. data). This high desiccation risk is due to the sandy soil in most parts of the forest. A variable time until hatching, in combination with the selection of spawning sites that assure that eggs or tadpoles come only into contact with water after ponds have completely filled, presumably offers tadpoles enough time to finish metamorphosis. When development continues within the eggs, tadpoles may additionally profit in competition with other tadpoles by their head start and towards smaller predators by their increased size (compare with RÖDEL, 1998).

According to STEWART (1967), another larger African ranid, *Rana fasciata fuelleborni*, deposits its eggs terrestrially. Single eggs or small groups are attached underneath overhang-

ing mats of sedges, other dense vegetation or on moist earth about 2.5 cm above water. Hatched tadpoles drop into water. A large number of West African forest frogs also deposit their eggs outside of water, e.g. various *Hyperolius* sp., all *Afrrixalus* sp., *Chiromantis rufescens* and all *Leptopelis* sp. (SCHIÖTZ, 1999; own data). In *Hyperolius*, *Afrrixalus* and *Chiromantis*, tadpoles are often washed into the ponds even after small rains that might not provide sufficient water to assure metamorphosis. Depositing clutches terrestrially at the border of potential ponds might minimize the risk of hatching too early. However, at these sites predation risk might be higher than in arboreal clutches that are often protected by plant parts (*Afrrixalus*) or foam (*Chiromantis*; but compare with RÖDFEL et al., in press *b*). In contrast to this assumption we never observed that *P. aequiplicata* eggs were eaten, even when driver ants were present, nor did we ever observe eggs covered with fungi. It might therefore be interesting to assay the egg capsules with respect to their chemical components.

#### ACKNOWLEDGMENTS

MOR was supported by a post-doctoral scholarship from the German Academic Exchange Service (DAAD). Analyzing and publication of the data was part of the BIOLOG program of the German Ministry of Education and Science (BMBF, Project W08 BIOTA-West, 01 LC0017). TROPENBOS-Côte d'Ivoire helped with transportation and various administrative services. Lodging facilities in TNP were provided by the "Centre de Recherche en Ecologie" and the "Projet Autonome pour la Conservation du Parc National de Tai (PACPNT)". The "PACPNT" and the "Tai Monkey Project" provided logistic support. Research permission was given by the "Ministère de l'Enseignement Supérieur et de la Recherche Scientifique" of the Republic of Ivory Coast. The access permit to TNP was issued by the "Ministère de la Construction et de l'Environnement". G. G. Gbamlin and C. Y. Ouoro were of invaluable help during field work. J.-L. Amet made valuable comments on a previous draft of the manuscript. These supports are gratefully acknowledged.

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Corresponding editor: Alain DUBOIS.

## Description of a new torrent frog in the genus *Arthroleptides* from Tanzania (Amphibia, Anura, Ranidae)

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**A new torrent frog in the genus *Arthroleptides* is described from the Udzungwa and Uluguru mountains in Tanzania. It is distinguished from the previously known species, *A. dutoiti* and *A. martiensseni*, by its large size, non-thickened supratympanic ridge, and more extensive webbing. A key to the three species in this endemic East African genus is presented.**

### INTRODUCTION

The series of block faulted highlands forming a chain on the eastern side of Tanzania are known collectively as the Eastern Arc Mountains (LOVETT, 1990). Major montane elements from south to north are the Udzungwas, Rubehos, Ukagurus, Ngurus, Ulugurus, East and West Usambaras, South and North Pares, and Taita Hills (fig. 1). The Eastern Arc Mountains are very old, consisting of basement crystalline rocks that were thrust up during the formation of the East African rift system (GRIFFITHS, 1993). It is believed that they have been biologically stable for long periods of time, during which many new species have evolved (LOVETT, 1993; FILLDSÅ & LOVETT, 1997). There is evidence that forest cover may have been in existence, continuously, for some 30 million years.

Forests on the Eastern Arc mountains are recognized for their diversity of plants and animals, and represent centers of high endemism in all groups of organisms studied (KINGDON, 1989; LOVETT, 1990).

A large proportion of the amphibians found in the forests of the Eastern Arc mountains is endemic. NUNN (1911) described a large torrent frog from the research station at Amani in

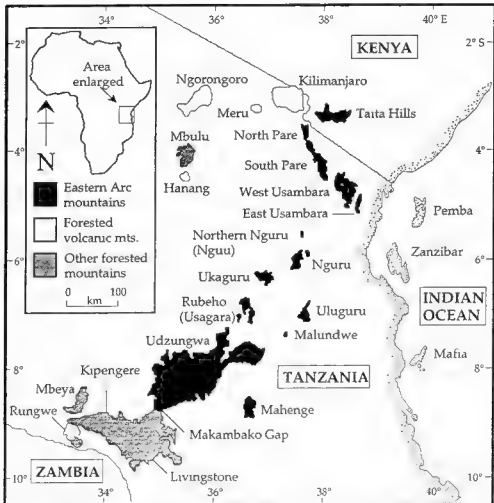


Fig. 1 Map showing the mountains comprising the Eastern Arc in Tanzania. *Arthroleptides yakusini* n. sp. is known from the Udzungwa and Uluguru mountains, and *A. martiensseni* is known from the West and East Usambaras and the Ngurus.

the East Usambaras. It was similar in many respects to the leaf litter frogs *Arthroleptis* Smith 1849, and the West African *Petropedetes* Reichenow, 1874. Nieden proposed the genus *Arthroleptis* for this frog. He named the species *Arthroleptis martiensseni*, after one of the collectors, the German military commander in Tanga. The type specimen of this species was collected at AMANI, and although the specimen presented by Martienssen to the Berlin Zoological Museum had no definite locality, Nieden assumed it was also from the Usambaras. This frog was distinguishable from all others in the area by the presence of large bifid discs on the fingers and toes. C. A. Du Toit discovered a second species of *Arthroleptis* on Mt Elgon



in 1934, that LOVERIDGE subsequently (1935) named *Arthroleptides dutoit*. It was distinguished from *A. martiensseni* by the dark ventral coloration with pale spots, smaller size, and eyes that protruded beyond the jaw when viewed from below.

*A. dutoit* is only known from Mt Elgon, an extinct volcano on the border between Kenya and Uganda. *A. martiensseni* has been recorded from the East Usambaras, the Ngurus, the Uluguru Mountains, and the Udzungwa Mountains in Tanzania, but fieldwork in Tanzania during 1999-2001 showed that torrent frogs from the Udzungwa and Uluguru mountain ranges were consistently separable from *Arthroleptides martiensseni* in the Usambaras. This southern form is here described as a new species.

Characters previously used to distinguish the species are reviewed, and a key is provided. KLEMENS (1998) described the extent of male secondary sexual characters in *Arthroleptides* based on a specimen from the Udzungwa Mountains. The specimen illustrated in that paper is selected as the type for the new species.

#### MATERIALS AND METHODS

Animals were collected in the field during 1999-2001, and photographed before being preserved. Comparative material was obtained from the collections in the Zoology and Marine Biology Department at the University of Dar es Salaam in Tanzania (UDSM and KMH numbers), the American Museum of Natural History (New York), the California Academy of Sciences (San Francisco), the Museum of Comparative Zoology at Harvard, and the Museo Tridentino di Scienze Naturali (Trento, Italy). Material with AC field numbers will be deposited in the American Museum of Natural History, specimens with RdS field numbers will be deposited in the National Museum in Washington, and specimens with ES field numbers will be deposited in the California Academy of Sciences in San Francisco. Specimens examined included tadpoles, juveniles, and large adults. The material examined is listed in app. 1, and includes 48 *Arthroleptides* n. sp. and two batches of *Arthroleptides* n. sp. tadpoles, 19 *A. martiensseni* and two batches of *A. martiensseni* tadpoles, and five *A. dutoit*. This genus is not well represented in collections.

The measurements taken, in millimeters, include: snout length, measured from anterior corner of eye to tip of snout, horizontal diameter of eye, horizontal tympanum diameter, distance from tympanum to snout tip; maximum jaw width; snout-vent length; distance between anterior corners of eyes, length of third finger to include the palmar tubercle, length of fourth toe to include the metatarsal tubercle, length of tibia measured with the leg bent; width of forearm. The measurements were compared as ratios of tympanum-snout/tympanum, snout-vent length/tympanum, width of disc of fourth toe/tympanum, jaw width/snout-vent length, tympanum/distance between anterior corners of eyes, tympanum/eye, snout-vent length/distance between anterior corners of eyes, tibia/snout vent length, snout-vent length/third finger, snout-vent length/ fourth toe, snout-vent length/eye, forearm width/eye, and jaw/tympanum. Webbing forms a thin margin along the toes, and traditional webbing formulae (e.g. SAVAGE & HEYER 1997) based on measures of phalanges free of web are not useful. However, the amount of webbing measured from the notch

between the toes ("main web") against the position of the proximal subarticular tubercle shows fixed differences between the species. The main webbing between the toes was measured against the proximal subarticular tubercles of the relevant toes. The main webbing of the fourth toe was measured on the inner and outer side.

Observations of breeding biology, including advertisement call, egg deposition sites and tadpole development were made for the new species at Kihansi in the Udzungwa mountains. Calls were recorded in the forest at 600 m in the Kihansi Gorge on the Udzungwa escarpment, using a Sony MZ-R70 recorder and an omnidirectional microphone placed at waist level in a bush near the calling individuals. A long cable permitted the observer to record the calls from five meters away. Calls were analysed using CANARY 1.2.4 (Cornell Laboratory of Ornithology, 1998) running on a Macintosh G4 or iBook. The tadpoles of the new species were compared to *A. martiensseni* tadpoles from the East Usambaras, and described using the approach of ALTIG & McDIARMID (1999).

#### *Arthroleptides yakusini* n. sp.

*Holotype*. – A male, collected on 8 December 1995 along the Njokomoni river, Udzungwa Mountains National Park, Tanzania, 07°48'57"S, 36°51'15"E, by M. W. Klemens. The specimen, AMNH A.151342, is housed in the American Museum of Natural History.

*Paratypes*. – Three specimens collected at the same time and place as the holotype, a male AMNH A.151343 and two females A.151341 and A.151344.

*Other material*. – Specimens assigned to this species are listed in app. 1

*Diagnosis*. – A large frog, exceeding 70 mm SVL in exceptional males, and 54 mm or over in 13 of 14 adults examined. The new species is distinguished from *Arthroleptides dutoti* by its larger size, over 35 mm SVL in mature males (less than 25 mm in *A. dutoti* males), webbing more extensive, with the main web reaching or passing the proximal subarticular tubercle on both sides of the fourth toe (never reaching in *A. dutoti*) ( $P < 0.0001$ , Mann-Whitney  $U$  test). The posterior edge of the supratympanic ridge is never thickened (always thickened in *A. dutoti*).

The new species is distinguished from *A. martiensseni* by more extensive webbing ( $P < 0.0001$ , Mann-Whitney  $U$  test) with the main web always reaching or passing the proximal subarticular tubercle of the fourth toe (only reaching in one specimen of 20 examined in *A. martiensseni*, falling far short in the other specimens).

*Description of the holotype*. – Comparative measurements of the holotype and the three paratypes are presented in tab. 1. The holotype is a male with well developed secondary sexual characters, 73 mm SVL (fig. 2). The head is broad, with the maximum jaw width (32.0) 44 % of the SVL. The eyes are not visible from below. The nostrils, situated near the front of the snout, open 45° postero-laterally. The tympanum (6.6) is dark, round, ringed with small tubercles. The snout to tympanum distance is 22.9. The horizontal diameter of the tympanum is slightly less than half the distance between the anterior corners of the eyes (13.5). The tympanic papilla is black, protruding from the upper half of the tympanum. The supratympanic ridge obscures the upper posterior margin of the tympanum. This ridge is nearly



Fig 2 Holotype of *Arthroleptis yakusini*, AMNH A 151342. Snout-vent length is 73 mm. Redrawn after KLEMENS (1998).

straight when viewed from the side, and is illustrated in KLEMENS (1998). The basal portion of the ridge is not thickened.

The forearm (10.7) is wider than the diameter of the eye (7.8). The terminal disks of the fingers and toes are large, bifid, with a pair of dorsal scutes. The width of the disk on the third finger is 50% of the width of the tympanum (fig. 3). The third finger is 20.4 measured to the base of the palmar tubercle. The palmar, metatarsal and subarticular tubercles are well developed. The tibia (43.7) is 59% of the SVL, and the fourth toe (38.9) is 53% of the SVL (fig. 4). No femoral glands are present.

Webbing extends in a distinct margin along the toes. The main web reaches the middle of the proximal subarticular tubercle of the first toe, the distal edge of the proximal subarticular tubercle of the second toe, and the lower inner edge of the proximal subarticular tubercle of the third toe, passing beyond the outer edge. The main webbing passes the proximal subarticular tubercle of the fourth toe on the inside, and reaches it on the outside. Main webbing reaches the middle subarticular tubercle of the fifth toe.

The dorsum and upper limbs are gray-brown with darker mottling. The back of the thigh is speckled with white on a dark background. The throat is dark with pale speckling, while the chest and belly have a paler background with white speckles. The underside of the limbs is pale. The dorsal skin is granular, with small white-tipped warts on the side of the head. The upper and lower jaws are edged with minute dark-tipped spines.

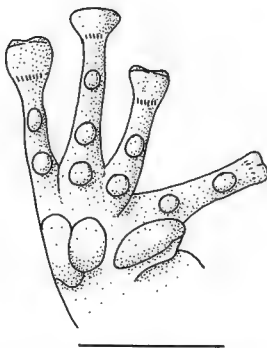


Fig. 3 Right hand (ventral view) of *A. yakusni* AMNH A 151342. Scale line 10 mm

The paratypes are similar but with none of the secondary sexual characters. See tab. 1 for comparative measurements.

The type and paratypes have encysted parasites in the skin, probably mites. These are also present in sympatric *Afrana* and *Phrynobatrachus* spp. in material collected at the same time.

*Other material examined.* The mean, range and standard deviation are given for the ratios investigated in tab. 2. The jaws are wide, varying between 40 and 45 % of the SVL in *A. yakusni*. The tympanum is exceptionally large in males displaying secondary sexual characters, with a dimension equal to nearly half the distance between the anterior corners of the eyes, but considerably less in smaller males and females, where the tympanum may be as small as 20 % of the distance between the anterior corners of the eyes. In large males showing spines on the chin and throat, and a ring of spines around the tympanum, the forearms are also hypertrophied, with the forearm width sometimes reaching up to 150 % the diameter of the eye. In animals not displaying secondary sexual characters, the forearm thickness is less than eye diameter, and may be as little as half eye diameter. The mean tibia/SVL proportion is 60 % in the three species, with no relationship with size or sex. The main web of *A. martenseni* never passes the subarticular tubercle of the fourth toe on either side. In only one specimen examined, a 19.8 mm SVL juvenile from the East Usambaras, did the main web just reach the proximal edge of the subarticular tubercle of the fourth toe, and just pass the subarticular tubercle of the third toe. In contrast, the main web of 31 of 34 *A. yakusni* examined passed

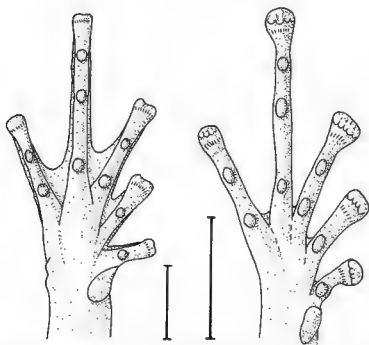


Fig 4 Right foot (ventral view) of *A. yakusui* AMNH A 151342 on the left and *A. martiensseni* CAS 168625 on the right. Scale lines: 10 mm.

the subarticular tubercle of the third toe, and reached or passed the subarticular tubercle of the fourth toe ( $P < 0.0001$ , Mann-Whitney  $U$  test). The proportions of the web and subarticular tubercles are such that there is nearly twice as much webbing in *A. yakusui* as in *A. martiensseni*. The digital discs vary by age and presumably by sex and reproductive status. Figure 5 illustrates the variation in the discs of the fourth toe in some specimens. The relative size of the disc, for example when compared to tympanum size, would not be useful in this genus. Work is in progress to investigate this variation. The body proportions investigated (tab. 2) show slight differences between males and females, but no significant differences between the three species.

The differences in the amount of webbing between *A. yakusui* and *A. martiensseni* are consistent in all specimens examined and represent an absolutely distinct character with no overlap in variation between the species.

In life, many individuals show an orange-reddish band through the upper part of the eye. The dorsal color pattern is a remarkably good camouflage against leaf litter on the forest floor. Smaller animals are nearly invisible against a wet log, for example, with only the pale digital scutes showing. Some smaller animals have distinct transverse banding on the back.

Table 1. – Comparative measurements of the holotype and paratypes of *Arthroleptides yakusini*.

Status AMNH number Sex	Holotype A 151342 Male	Paratype A 151341 Female	Paratype A 151343 Male	Paratype A.151344 Female
SVL	73	57	55	56
Eye	7.8	7.2	6.2	7.1
Tympanum	6.6	3.1	4.5	3.5
Tympanum-snout tip	22.9	19.5	17.6	18.7
Jaw width	32.0	23.6	22.8	24.0
Anterior eyes	13.5	12.2	10.8	11.4
Length 3 <sup>rd</sup> finger	20.4	16.9	16.3	17.3
Forearm width	10.7	4.0	5.5	4.7
Length 4 <sup>th</sup> toe	38.9	32.0	28.4	30.8
Tibia	43.7	34.3	34.2	37.2
Main inner web toe 4: proximal tubercle	Passes	Passes	Passes	Reaches
Main outer web toe 4: proximal tubercle	Reaches	Reaches	Passes	Reaches

Table 2. Mean, range and standard deviation for the ratios examined in specimens of *Arthroleptides yakusini*. The overall mean is based on males, females and juveniles.

Ratio	Overall mean	Males Mean (range) s, n	Females Mean (range) s, n
Tympanum-snout / tympanum	5.64	4.36 (3.81-5.62) 0.84, 7	5.64 (4.86-6.29) 0.47, 6
Snout-vent length / tympanum	16.59	13.11 (10.80-17.08) 2.53, 7	16.63 (16.00-18.48) 0.96, 6
Jaw width / snout-vent length	0.43	0.43 (0.40-0.45) 0.02, 7	0.43 (0.41-0.44) 0.01, 6
Tympanum / anterior eyes	0.31	0.39 (0.27-0.49) 0.08, 7	0.31 (0.25-0.32) 0.02, 6
Tympanum / eye diameter	0.48	0.73 (0.73-0.85) 0.06, 4	0.48 (0.43-0.56) 0.05, 5
Snout-vent length / anterior eyes	4.92	5.01 (4.37-5.63) 0.43, 7	4.92 (4.70-5.19) 0.18, 6
Tibia / snout-vent length	0.63	0.65 (0.60-0.68) 0.03, 7	0.63 (0.59-0.69) 0.04, 6
Snout-vent length / third finger	3.50	3.29 (3.14-3.58) 0.17, 7	3.50 (3.24-3.56) 0.12, 6
Snout-vent length / fourth toe	1.86	1.77 (1.61-1.93) 0.10, 7	1.86 (1.72-2.01) 0.10, 6
Snout-vent length / eye	7.70	9.07 (8.85-9.36) 0.34, 3	7.71 (7.68-8.99) 0.52, 5
Forearm / eye	0.68	0.95 (0.89-1.37) 0.23, 4	0.68 (0.56-0.94) 0.17, 5
Jaw width / tympanum	7.05	5.67 (4.85-7.25) 1.00, 7	7.06 (6.62-7.61) 0.39, 6

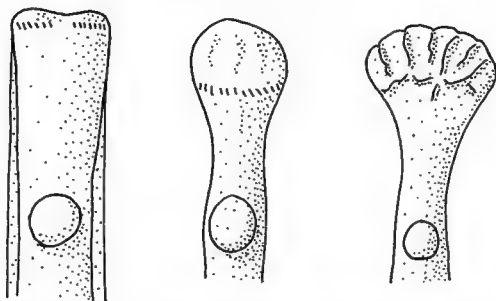


Fig 5. Comparison of the discs of the fourth toe of *A. yakusini* AMNH A 151342 (left), *A. martiensseni* CAS 168625 (middle) and *A. yakusini* KMH 21534 (right). Similar variation was found within all populations examined.

**Male secondary sexual characters.** KLEMENS (1998) discussed the development of male secondary sexual characters in *Arthroleptides*. These are shared by its assumed sister group, *Petropedetes*, in West Africa. The characters include a soft tympanic papilla, spines on the chin and throat, enlarged forearms and a ring of spines around the tympanum. Unlike most frogs in the area, it appears that the males are larger than the females. Males collected out of season, smaller males and females show none of these characters (KLEMENS, 1998). The relationship between *Petropedetes* and *Arthroleptides* is under investigation. NARINS et al. (2001) investigated the function of the tympanic papilla in *Petropedetes parkeri*.

**Advertisement call.** Males call after the start of the short rains in November. Calling takes place after dark, from the forest floor, although males may climb on to rocks or fallen tree trunks to call. The call is a series of short "nauks" repeated at long intervals of up to 25 seconds. A total of 39 calls from five individuals was analysed. Calls consist of 4 to 8 pulses (mode 6) produced in 30.6 to 81.2 ms ( $\bar{x}$  60.9 ms,  $s$  9.1,  $n$  39). The resulting pulse rate varies from 73.2 to 106.5/s ( $\bar{x}$  85.7/s,  $s$  8.5,  $n$  39). The emphasized frequency varies from 1.2 to 1.4 kHz ( $\bar{x}$  1.3 kHz,  $s$  0.04,  $n$  39). A sound spectrogram of a typical call is illustrated in fig. 6.

**Eggs.** The following descriptions are based on observations made in January 2001 at Kihansi. The dark eggs are deposited either in small groups of four or five, or in large masses of about 200. The eggs are always attached to sloping or vertical rock faces that are covered by a film of water. Near the base of the Kihansi falls the rocks were wet from drifting spray, while

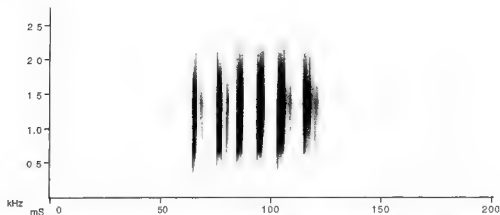


Fig. 6 - Sound spectrogram of a typical advertisement call of *Arthroleptides yakusini*, Kihansi Gorge, Udzungwa mountains, Tanzania. 24°C, 28 January 2001.

other rocks in dryer situations were wet from natural drainage from higher ground. Each egg is 2 mm in diameter, in a 4 mm capsule. The eggs are camouflaged against the dark reflective surface.

*Tadpoles.* Tadpoles at various stages were collected over a period of three months from the Udzungwa escarpment. They are similar in body proportions and mouthparts to the tadpoles of *Arthroleptides martensseni* (DREWES et al., 1989, whose descriptive approach is followed here for comparison).

This description is based on a tadpole of stage 37 (AC 2452) collected in the Kihansi Gorge in the Udzungwa Mountains, Tanzania. The individual (fig. 7) has a total length of 27.4. Its other measurements and major features are: body length 8.7, tail length 18.7, body height 6.0 from snout 3.1, body width 6.0 from snout 4.7, tail muscle height at base 1.5, greatest dorsal fin height 10.0 from tail tip. The ventral fin is absent. Interorbital distance 2.3, internarial distance 1.4, snout to anterior edge of nares 0.8, snout to anterior corner of eye 1.9, snout to spiracle 5.2, posterior edge of nares to anterior corner of eye 1.0, naris diameter 0.13, eye diameter 1.3, pupil diameter 0.6 and width of oral disc 2.2. Oral disc without anterior oral papillae (fig. 8), vent medial, eyes protruding dorsally, sinister spiracle, with a labial tooth formula 3(1-3)/3(1).



Fig. 7 - A 27.4 mm tadpole of *Arthroleptides yakusini* from Kihansi, Udzungwa Mountains, Tanzania AC 2452



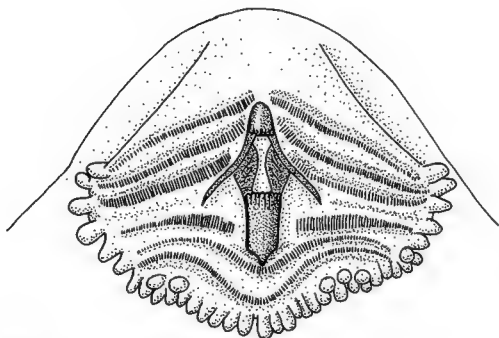


Fig. 8 - Oral disc of the tadpole of *A. yakusni* AC 2452

The labial tooth rows have about 52 teeth/mm. The upper jaw sheath is strongly curved, resembling the beak of a parrot. The lower jaw sheath is acutely curved, fitting inside the upper sheath when the jaws close. The posterior edge of the oral disc has a double row of marginal papillae, becoming single towards the angle of the jaw. The papillae are short and rounded.

The oral disc is closed in preserved specimens, similar to the illustration in DREWES et al. (1989). The upper labium is flexed to cover the lower labium. When the disc is closed the medial ends of labial tooth rows A-1 overlap.

The body is widest posteriorly in dorsal view. The dorsal fin is very reduced, as high as it is wide. The ventral fin is absent. In an earlier stage 23 tadpole (fig. 9) both fins are better developed. At this stage the external gills are still present. In specialised tadpoles like these that live on wet rocks, the timing of the loss of external gills and the development of hind legs



Fig. 9 - A stage 23 tadpole of *Arthroleptodes yakusni* from Kihansi, Udzungwa Mountains, Tanzania, AC 2453, to show the dorsal and ventral fin development

for locomotion are not comparable to more typical tadpoles and it is not possible to compare stages directly. The eyes protrude dorsally. The spiracle opening is a small vertical slit 0.1 high. The vent tube is rounded, extending 0.5 beyond the hind legs, the opening not attached to the tail muscle. Femoral glands are present extending from near the knee, for 1-3 along the back of the thigh. The toes are separate with distinct discs. The bifid dorsal disc scutes are not yet developed.

The dorsal surface is dark with darker markings and pale spots. The dorsal half of the tail is much darker than the lower half. Nine light dorsal patches on the tail extend on to the sides. These are saddle-like in living specimens. Ventrally the belly has some mid-belly speckling with fine spots. All other ventral surfaces are white. Five gut spirals are just visible through the belly muscles.

Tadpoles hatch from the eggs and remain on the wet rock faces. They may occur at high densities on rock faces in a film of water, with 22 counted in one square meter below the Uluguru North Forest Reserve, grazing on algae. They complete development in the film of water, taking 8 to 10 weeks. Tadpoles were found during the day under stones in a road-side ditch at Morningside, Uluguru Mts (1 May 1980). After the tail is resorbed, juveniles leave the nursery rock and can be found feeding in vegetation along streams and in leaf litter in forest.

*Etymology.* The species name *yakusini* is an adjective, derived from the Swahili *ya kusini*, meaning "of the south". It refers to the distribution of this species in the mountains to the south of the range of *A. martiensseni*, with which it has long been confused.

#### DISTRIBUTION AND HABITAT OF THE THREE SPECIES

The three species of *Arthroleptides* are allopatric as far as is known, but further fieldwork is needed to determine their detailed distributions. *Arthroleptides datoiti* is known from rocky streams that drain Mt Elgon, an extinct volcano on the common border of Kenya and Uganda. Much of the forest habitat on the lower slopes has been cleared and it has been suggested that this species is extinct (DREWES et al., 1989). However, these seasonally common frogs should be looked for at the start of the rains. They are found in cracks and under large rocks on the side of steep slopes, a habitat requiring a lot of search-effort. Their eggs are large (3 mm) and pigmented, but the tadpoles are unknown.

*Arthroleptides martiensseni* is found in the East and West Usambaras, and Nguru mountains. It occurs both in forest leaf litter far from water, and along rocky streams. Juveniles have been found in quiet pools and seepages. The adults shelter under large boulders along fast flowing streams, emerging after nightfall to feed. The wide jaws suggest that this species and *A. yakusini* are important predators on smaller frogs like *Arthroleptis stenodactylus* Pfeffer, 1893 that are common in the forest leaf litter. The eggs are unknown, but the tadpoles of *A. martiensseni* with very characteristic mouthparts have been described (DREWES et al., 1989).

*Arthroleptides yakusini* is known from the Uluguru and Udzungwa mountains from 300 m up to 2800 m. After the tadpoles metamorphose, the juveniles leave the nursery rock and move into vegetation and leaf litter to feed. They have been found in small pools along

streams, or climbing on leaves on the top of vegetation. Small, non-adult individuals of *A yakusmi* are also found in cracks and fissures of vertical rock and soil faces along small streams and drying stream beds. They peer out at observers and immediately try to go deeper in if disturbed. The adults, especially the larger animals, move deep into cracks under large rocks during the day, emerging onto wet rock faces after dark to feed. Adults have been found on the forest floor some distance from water, under a rotting log on a stream bank, and occupying a crack in tree bark. Sometimes an individual will emerge and sit out on rocks just covered with shallow, fast flowing water, where it can easily escape downstream with the current. Adults are capable of large leaps.

### IDENTIFICATION

The characters used by LOVFRIDGE (1935) to distinguish between *A dutoitii* and *A martiensseni* (eyes visible from below, and dark belly with white spots) are of limited use. The jaws are relatively wider in larger animals, and the eyes may be visible from below in smaller specimens of *A martiensseni* and *A yakusmi*. All three species have individuals with purple bellies and white spots. The following key will help identifying species of *Arthroleptides*:

- 1a. Base of glandular supratympanic ridge thickened, animals never over 35 mm SVL . . . . .  
     . . . . . *Arthroleptides dutoitii*
- 1b. Base of supratympanic ridge never thickened, mature animals exceeding 50 mm SVL  
     . . . . . 2
- 2a. Web notch reaching or passing proximal subarticular tubercle of fourth toe . . . . .  
     . . . . . *Arthroleptides yakusmi*
- 2b. Web notch never reaching proximal subarticular tubercle of fourth toe . . . . .  
     . . . . . *Arthroleptides martiensseni*

### ACKNOWLEDGEMENTS

The National Geographic Society is thanked for providing grant 6475 to AC. The Wildlife Conservation Society of New York provided support through Michael W. Klemens who also provided illustrative material. Jenny Channing, Alison Channing, Billy Munisi, Elia Mulungu and Rafael de Sa are thanked for assistance in the field. The Tanzanian Commission for Science and Technology COSTECH issued a research permit 99/55 NA-99-40 to AC for which they are thanked. Jenny Channing prepared figs 2, 7 and 9. The National Research Foundation of South Africa assisted financially and much of the work was undertaken during a sabbatical granted to AC by the University of the Western Cape.

The following kindly loaned comparative material in their care: Dr I. Ford (AMNH), Jens Rasmussen and Dr R. C. Drewes (CAS), Jose Rosado and Dr J. Hanken (MCZ), M. Menegon (MTSN). We acknowledge Frontier Tanzania, a joint cooperative research programme between the Society for the Exploration of the Environment, UK and the Faculty of Science, University of Dar es Salaam who collected some of the material.

Dr J. C. Poynton offered valuable advice on an earlier draft.

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## APPENDIX 1

## LIST OF SPECIMENS EXAMINED

The following abbreviations are used

- AC Field numbers of A. Channing. Material is to be deposited in the American Museum of Natural History
- AMNH. - American Museum of Natural History, New York.
- CAS. - California Academy of Sciences, San Francisco.
- ES. Field numbers of E. Scott. Material will be deposited in the California Academy of Sciences.
- KMH. Field numbers of K. M. Howell, specimens in the collection of the Zoology & Marine Biology department, University of Dar es Salaam
- MCZ. - Museum of Comparative Zoology, Harvard
- MTSN. - The Museo Tridentino di Scienze Naturali (Trento, Italy)
- RdS. Field numbers of R. de Sa. Specimens will be deposited in the National Museum, Washington, DC
- UDSM. The University of Dar es Salaam, collection of the Zoology and Marine Biology department

*Arthroleptides ditotti*

Five specimens from Mount Elgon, Kenya. AMNH A 68670, A 68673, A 68675, A 68677, 68678

*Arthroleptides martiensseni*

Altogether 19 frogs and two batches of tadpoles.

East Usambara Mountains, Tanzania. Females USDM 1107, CAS 168625, 168633, 168684, AC 2234; ES 705; ES 723. Males: CAS 168627, 168631. Juveniles AC 1905, 2045.

Segoma Forest Reserve, East Usambara Mountains, Tanzania - Male: KMH 17503. Juvenile: KMH 17549

Amani, East Usambara Mountains, Tanzania - Females MCZ A.12823 Males. MCZ A 12824.

Mt Lutind, East Usambara Mountains, Tanzania. - Female: MCZ A.12825.

Magrotto Mountain, East Usambara Mountains, Tanzania. Males. MCZ A.25380-81 Juvenile MCZ A 25382.

Zigi river, East Usambara Mountains, Tanzania. Tadpoles. CAS 168615, 168617

*Arthroleptides yakusini*

A total of 48 frogs and two batches of tadpoles

Njokomoni River, Udzungwa Mountains National Park, Tanzania - Males: AMNH A.151342 (holotype), A 151343 (paratype) Females AMNH A 151341, A 151344 (paratypes).

Kihansi Gorge, Udzungwa Mountains, Tanzania. - Juvenile: UDSM 1038. Tadpoles. AC

Udzungwa Mountains, Tanzania - Males: UDSM 1116, 1138-39 Juveniles. AC 1930, UDSM 1038 (9 specimens).

Kihansi Gorge, Udzungwa Mountains, Tanzania. Juvenile AC 2379

Kitolomero, Udzungwa Mountains, Tanzania Juveniles. MTSN unnumbered (20 specimens).

Kihanga, Udzungwa Mountains, Tanzania Females: MTSN unnumbered (2 specimens)

Tegetero, Uluguru Mountains, Tanzania. Male RdS 862. Juveniles RdS 849, 866

Morningside, Uluguru Mountains, Tanzania. - Tadpoles: CAS 159944.

Bagilo, Uluguru Mountains, Tanzania. Males AMNH A 37281, MCZ A 12817, A.12820.

Vituri, Uluguru Mountains, Tanzania. - Female: MCZ A.12822

*Corresponding editor:* Alain DUBOIS

**A new species of *Adenomera*  
(Anura, Leptodactylidae)  
from the *Araucaria* forest  
of Rio Grande do Sul (Brazil),  
with comments on the systematic status  
of southern populations of the genus**

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**We describe a new species of *Adenomera* from Rio Grande do Sul, southern Brazil. This new species inhabits the spiny understory of southern *Araucaria* forests, and can be most easily distinguished from other *Adenomera* by its small body size, unexpanded toe tips and distinctive advertisement call. Calls are analyzed, described, and compared to advertisement calls of other *Adenomera* of southern Brazil, and the systematic status of these populations is discussed.**

## INTRODUCTION

Extensive morphological variation both within and among populations of species of the leptodactylid genus *Adenomera* have traditionally rendered this a taxonomically difficult group. The potential use of advertisement call data as a means of resolving this group's systematics had already been suggested by HEYER (1984) and is being effectively used in resolving species identity in member species distributed throughout the Amazon Basin (ANGULO & ITOCHEA, in press; ANGULO et al., in press). Two species, *Adenomera marmorata* Steindachner, 1867 and *A. bokeri mami* (Heyer, 1973), have been reported to occur in sympatry in southeastern Brazil (HEYER, 1973). Although these two nominal species have long been

suspected to be composites of two or more species (HEYER, 1977, 1984), it has not been until recently that enough acoustic data from different localities were collected to determine species identities within the group.

KWET (1998) reported the southernmost record for the genus in the Brazilian state of Rio Grande do Sul. This population, which was termed *Adenomera* cf. *marmorata*, has distinct advertisement calls compared with those from populations in the states of São Paulo, Rio de Janeiro and Santa Catarina. Comparison of specimens from Rio Grande do Sul with specimens from other localities in southeastern Brazil, including type material of the nominal species and their synonyms, revealed that there are also morphological differences associated to this different call. Based on this evidence, we conclude that the Rio Grande do Sul population comprises a new species of *Adenomera* and proceed to describe it herein.

### MATERIALS AND METHODS

Individuals of the new species were collected in Rio Grande do Sul, at Serra Geral between 600 and 1000 m altitude, and later deposited in the Museu de Ciências e Tecnologia da PUCRS (MCP, Porto Alegre, Brazil). Specimens examined for comparisons are deposited in the Museu de Ciências e Tecnologia da PUCRS (MCP, Porto Alegre, Brazil), Naturhistorisches Museum Wien (NHW, Wien, Austria), Staatliches Museum für Naturkunde Stuttgart (SMNS, Stuttgart, Germany), National Museum of Natural History (USNM, Washington, D.C., USA), Museum für Naturkunde Berlin (ZMB, Berlin, Germany), Zoologisches Museum Hamburg (ZMH, Hamburg, Germany) and Zoologische Staatssammlung München (ZSM, München, Germany). Our specimens were fixed in 6% formalin and preserved in 70% ethyl alcohol. All measurements were made with digital calipers. The following measurements were made to the nearest 0.1 mm: snout-vent length (SVL), head length, defined as the diagonal distance from the tip of the snout to the right angle of the jaw (HL), head width, defined as the distance between the angles of the jaw (HW), horizontal eye diameter (ED); horizontal tympanum diameter (TD), eye-nostril distance (END); interorbital distance (IOD); internarial distance (IND), thigh length (THL); tibia length (TL), foot length (FL).

Field work took place from 1995 to 1999 (in each year from about October to March), at the Centro de Pesquisas e Conservação da Natureza Pró-Mata (CPCN) (29°30'S, 50°10'W), municipality of São Francisco de Paula, state of Rio Grande do Sul, Brazil. Acoustic signals were recorded using a Sony WM-D6C tape recorder, a Sennheiser microphone system K6 with ME66 module, and metal tapes Sony Metal XR-90. Advertisement calls of four males of the new species were recorded at CPCN Pró-Mata. (a) on 8 November 1995, 23:30 h, 19°C air temperature, (b) on 20 December 1995, 14:00 h, 26°C air temperature, (c, d) on 1 November 1996, 3:00 h, 16°C air temperature. These calls are unvouchered. Acoustic analysis was performed using a Macintosh based digital signal analysis software, Signalyze 3.12 (KLEFFER, 1994), at a sampling rate of 44.1 kHz and 16 bit precision. Temporal and spectral signal figures were produced using a combination of Signalyze 3.12, DADiSP, a PC-based sound analysis software, and Corel Draw. Measurements of six acoustic parameters were taken: call length, call rate, call rise time (time from beginning of call to peak amplitude), dominant frequency, other frequencies with perceptible energy and frequency modulation (measured as

the difference between final frequency and initial frequency). Temporal parameters were measured in milliseconds (ms) and spectral parameters in Hertz (Hz). Dominant frequency measurements were taken at the peak amplitude of each call.

## RESULTS

### *Adenomera araucaria* sp. nov.

(fig. 1-2)

*Holotype*. MCP 2421, adult male, collected at the Centro de Pesquisas e Conservação da Natureza Pró-Mata (CPCN) (about 950 m a.s.l.), 29°30'S, 50°10'W, municipality of São Francisco de Paula, Rio Grande do Sul, Brazil, on 20 December 1996, by Axel Kwet and Marcos Di-Bernardo.

*Paratypes*. Nine specimens collected at the type locality by Axel Kwet and Marcos Di-Bernardo (MCP 1794, female, 19 December 1995, MCP 1849, subadult, 9 February 1996; MCP 3208, male, 19 March 1997; MCP 3209, subadult, 23 November 1997; MCP 3463, male, 20 December 1996; MCP 3672-73, males, 24 November 1998, MCP 3676, male, 11 January 1999, MCP 3677, female, 4 January 1999); two specimens collected near Encruzilhada das Antas, municipality of Bom Jesus, Rio Grande do Sul, by Axel Kwet and Marcos Di-Bernardo (MCP 3345-46, males, 4 January 1998).

*Diagnosis*. – A small sized *Adenomera* (maximum SVL 18.8 mm in males, 19.9 mm in females; tab. 1) with toe tips rounded or slightly swollen, not disked (stage B after HEYER, 1973). *Adenomera araucaria* differs from all other known species in the genus by its advertisement call. Morphologically, it is most similar to *Adenomera marmorata*, being distinguished from this species by: (1) toe tips not flattened, (2) white tubercles on tarsus and on sole of foot less developed, (3) shorter limbs; (4) snout more acuminate in dorsal and lateral view; and (5) ventral surface of thigh smooth, whitish, only sparsely dotted with gray (partly granular and dark mottled, often forming a net-like pattern in *A. marmorata*). Whereas the new species differs from northern populations of *A. marmorata* also by a smaller size (maximum SVL near the type locality about 24 mm in males and 25 mm in females, HEYER, 1973), specimens from southern populations of *A. marmorata* (which may be a distinct species) are equally sized (maximum SVL of *A. marmorata* at the type locality of *Leptodactylus nanus* Müller, 1922: 19.3 mm in males, 20 mm in females, tab. 1). *Leptodactylus trivittatus* Lutz, 1926, is considered synonymous with *A. marmorata*, having toe tips distinctly expanded.

*Adenomera araucaria* differs from *Adenomera bokermanni* (following the description of HEYER, 1973, but see discussion) by: (1) its smaller size (maximum SVL of *A. bokermanni*, 25.1 mm in males, 27.6 mm in females); (2) absence of perceptible white tubercles on tibia; and (3) distinct dorsal pattern, usually with longitudinal arranged dark marks and light dorsolateral and mid dorsal stripes (dorsum commonly uniform or with indistinct marks in *A. bokermanni*). The new species is distinguished from all other known species of the genus, i.e., *Adenomera andreae* (Müller, 1923), *Adenomera dipti* (Boettger, 1885), *Adenomera hvlaedactyla* (Cope, 1868), *Adenomera lutzi* Heyer, 1975 and *Adenomera martinezi* (Bokermann, 1956) by its smaller body size. Further, it differs from nominal *A. andreae* and *A. lutzi* by non expanded toe tips, from *A. dipti* and *A. hvlaedactyla* by less rugose skin texture and from *A.*



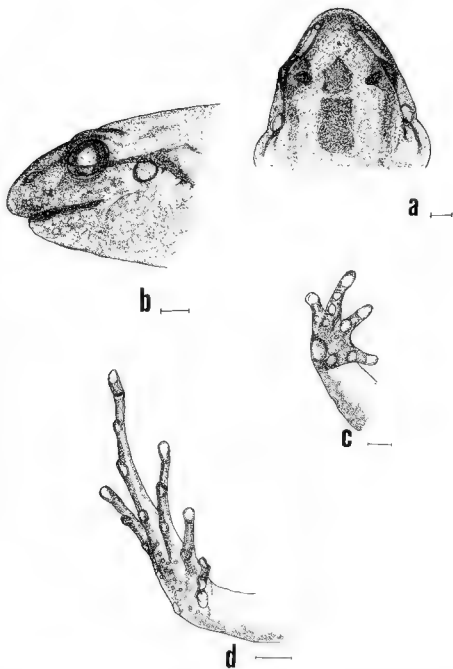


Fig 1 *Adenomera araucana* sp nov, holotype male, MCP 2421 (a) Lateral and (b) dorsal views of head; ventral views of (c) right hand and (d) right foot. Scale bar: 1mm

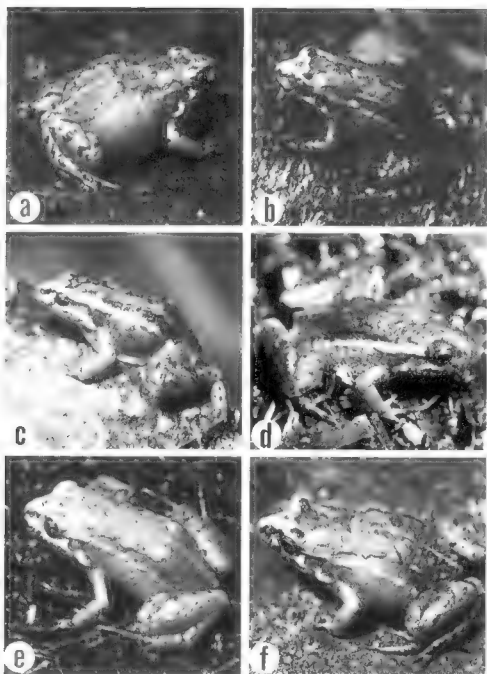


Fig 2 *Adenomera araucana*, type specimens in life showing intraspecific variation. (a) MCP 2421 holotype, male; (b) MCP 1794, paratype, female; (c) MCP 3346, paratype, male; (d) MCP 3208 paratype, male; (e) MCP 3676, paratype, male; (f) MCP 3463, paratype, male.

Table 1 -- Morphometric characters for *Adenomera araucaria* from Serra Geral, Rio Grande do Sul, *Adenomera cf. araucaria* from Morro do Baú, Santa Catarina, and *Adenomera cf. marmorata* from Corupá, Santa Catarina (type locality of *Leptodactylus namus*) *n*, sample size; *s*, standard deviation

	<i>Adenomera araucaria</i> Serra Geral, RS						<i>Adenomera cf. araucaria</i> Morro do Baú, Ilhota, SC					<i>Adenomera cf. marmorata</i> Corupá, SC					
	Males ( <i>n</i> = 8)			Females ( <i>n</i> = 3)			Male ( <i>n</i> = 1)	Females ( <i>n</i> = 5)			Males ( <i>n</i> = 5)			Females ( <i>n</i> = 7)			
	Range	Mean	<i>s</i>	Range	Mean	<i>s</i>		Range	Mean	<i>s</i>	Range	Mean	<i>s</i>	Range	Mean	<i>s</i>	
Snout-vent length	17.1-18.8	18.4	0.59	19.3-19.9	19.6	0.31	17.9	18.6-20.0	19.2	0.52	17.1-19.3	18.4	0.92	17.3-20.0	18.8	0.90	
Head length	5.9-6.5	6.2	0.20	5.7-6.9	6.2	0.61	5.7	5.8-6.8	6.4	0.44	5.5-6.2	5.9	0.26	5.9-6.3	6.0	0.14	
Head width	5.5-6.7	6.0	0.37	5.9-6.0	6.0	0.06	5.6	5.6-6.5	6.0	0.41	5.4-6.1	5.4	0.28	5.7-6.4	6.1	0.28	
Eye diameter	1.7-1.9	1.8	0.07	2.0-2.0	2.0	0	1.9	1.9-2.1	2.0	0.08	1.9-2.0	1.9	0.05	1.8-2.0	1.9	0.10	
Tympanum diameter	1.1-1.3	1.2	0.07	1.3-1.3	1.3	0	1.1	1.1-1.3	1.2	0.08	1.0-1.2	1.0	0.08	0.9-1.4	1.1	0.17	
Eye-nostril distance	1.4-1.7	1.6	0.09	1.4-1.7	1.6	0.15	1.6	1.5-1.9	1.7	0.18	1.4-1.7	1.4	0.11	1.6-1.8	1.7	0.08	
Interorbital distance	3.4-3.9	3.6	0.20	3.4-3.7	3.5	0.17	3.4	3.3-3.7	3.5	0.16	3.2-3.7	3.2	0.23	3.2-3.8	3.5	0.23	
Internarial distance	1.8-2.2	2.0	0.14	1.9-2.0	1.9	0.06	2.0	1.9-2.0	2.0	0.05	2.0-2.3	2.0	0.13	1.8-2.2	2.0	0.13	
Femur length	7.3-8.2	7.8	0.35	7.8-8.1	8.0	0.15	8.4	8.2-8.9	8.5	0.26	7.5-8.8	7.5	0.50	8.2-9.6	8.7	0.51	
Tibia length	7.6-8.3	7.9	0.27	8.0-8.8	8.4	0.40	8.7	8.5-9.1	8.8	0.25	7.6-8.9	7.6	0.48	8.2-9.7	8.9	0.53	
Foot length	9.0-9.9	9.4	0.33	9.8-10.1	10.0	0.15	9.4	9.6-10.5	10.1	0.37	8.8-9.9	8.8	0.47	9.3-10.4	9.8	0.35	

*martinezi* by dorsal pattern not consisting of four symmetrically arranged rows of longitudinal spots. *Adenomera araucaria* is currently known only from the southernmost extent of the Atlantic Forest Domain in southeastern Brazil.

*Description of holotype* Body small, robust, with short limbs (tab 1). Head as long as broad, dorsal outline of snout subelliptical, slightly acuminate (fig. 1a); snout profile subacuminate (fig 1b). Nostrils directed dorsolaterally, closer to tip of snout than to eye; internarial distance about one third of head width. Tympanum distinct, its diameter two thirds of eye diameter, supratympanic fold poorly developed, highlighted with black. Canthus rostralis indistinct, rounded. No cranial crests. Angle of jaw with white oval gland. Vocal sac single, internal. Paired, elongate vocal slits in males. Vomerine teeth in short transverse series, posterior to choanae and separated from each other by about the length of one tooth row. Arms and fingers relatively short. Finger lengths  $III > I = II > IV$ ; finger tips rounded, not expanded, fingers without webbing or fringes (fig. 1c). Two large, ovoid metacarpal tubercles; size of outer metacarpal tubercle about two times inner metacarpal tubercle; prominent, rounded subarticular tubercles on fingers. Nuptial asperities absent. Hindlegs short, tibia a little longer than femur. Toe lengths  $IV > III > V > II > I$ ; toe tips slightly swollen but not noticeably expanded nor flattened, toes without webbing or fringes (fig. 1d). Distinct, ovoid metatarsal tubercles and rounded subarticular tubercles; inner metatarsal tubercle slightly larger than outer metatarsal tubercle. Distinct inner tarsal fold. Sole of foot and lower surface of tarsus with small, but relatively distinct tubercles. Upper shank surface smooth, without perceptible white tubercles. Dorsal texture smooth, except some tubercles around vent (on posterior region of thighs and on sacral region) and, dorsolaterally, two longitudinal glandular folds. A distinct lateral fold partly separated into small glandular segments extending from eye, over tympanum and shoulder, to inguinal region. Above this fold a second, less distinct dorsal fold from shoulder to sacral region. Ventral surface smooth.

*Color in life.* – Dorsal coloration orange-brown, with a symmetrical pattern of dark marks (fig. 2a). A black, pentagonal interorbital spot followed by a larger rectangular blotch between shoulders (fig. 1a) and another spot on anterior part of dorsum. A black stripe on each dorsal glandular fold joined on middorsum by a black bar, and a light stripe on each lateral fold. On posterior half of body, a light mid-dorsal stripe. A black line from nostril to anterior margin of eye; another line from posterior margin of eye, over tympanum and a white gland on angle of jaw, to insertion of arm. Black, irregular cross-bars on hindlimbs and partly on arms. Belly white, immaculate, skin on throat and ventral surface of thigh very sparsely dotted with small melanophores.

*Color in preservative* In 70% alcohol, dorsum brownish with the dorsal pattern of dark marks described above. Venter white, immaculate.

*Measurements of holotype* (in mm) SVL 18.1, HL 5.9, HW 5.9, ED 1.8, TD 1.2; END 1.6, IOD 3.5; IND 1.9; THL 7.3; TL 7.6; FL 9.3.

*Variation.* Females slightly larger than males (tab 1). Dorsal outline of snout subelliptical or nearly acuminate in males but more rounded in females. In all specimens, inner metacarpal tubercle smaller than outer metacarpal tubercle, in size varying between one half and two thirds. Dorsal coloration very variable, consisting of various shades of light or dark brown, orange-brown, tan or gray, with a usually inconspicuous pattern of longitudinally arranged, irregular, dark marks (fig. 2, see color photos in KWEI & DI-BERNARDO, 1999). Variable,

black, triangular or hourglass-shaped interorbital spot from between upper eyelids to region between shoulders. Light middorsal stripe usually extending from above vent to middle of body not reaching head; this stripe very weak in one of the 12 type specimens (MCP 3676). Dorsolaterally, most specimens with two black longitudinal lines and below with two finer, light stripes, but in two specimens (MCP 3208, 3346), these stripes expanded into broad, white lateral lines. Two specimens (MCP 3673, 3677) with dark dorsal coloration and pattern hardly visible. Ventral surface of body usually white, finely dotted with gray on throat and posterior part of thighs, but one specimen (MCP 3209) very dark ventrally, with a lot of melanophores on lower thighs, throat and belly

**Advertisement call** Acoustic parameter measurements of four specimens are listed in tab 2; fig 3 depicts oscillogram, spectrum, and sonogram images of the call. The call length of the new species ranges from 85.5 to 139.5 ms in duration and call rate is relatively slow (26.2 to 44.8 calls/min). Call onset is gradual and call rise time varies from 35.1 to 81.0 ms, constituting approximately 26.8 to 66.6 % of the total call duration. The call does not possess perceptible pulses, although it does show some amplitude modulation that can take the form of 5-11 severe amplitude modulations on the amplitude envelope. The call is a frequency-modulated signal, with an upward frequency sweep where the end of each call has a higher frequency than the respective beginning of the call. This frequency sweep is audible to the human ear and varies from 775.3 to 1808.8 Hz. The fundamental frequency or first harmonic lies between 1722 and 3359 Hz and the main carrier frequency oscillates between 4625 and 5403 Hz, comprising the second harmonic band. Other frequency components which have some energy lie approximately at 5943-8613 Hz and 8354-10938 Hz. A much higher harmonic band also presents perceptible energy, greater than all other aforementioned frequencies, except for the carrier, between 13716 and 15231 Hz. However, the energy peaks of any of these frequencies are substantially lower than the main carrier, making the call almost a pure tone (see spectrum on fig. 3); other frequencies are not depicted as their energy levels fall below the cutoff point of -30 dB. The number of frequencies detected at any one time in calls varies from three to six, although this may vary with recording conditions.

**Natural history** – The new species inhabits subtropical rainforest of northeastern Rio Grande do Sul and southern Santa Catarina, from about 300 to 1100 m altitude. It was very abundant in secondary forests and *Araucaria* stands on the eastern Planalto of Rio Grande do Sul. Most specimens were found near trunks of the Paraná pine, *Araucaria angustifolia*, where they may find protection against potential predators by hiding among the spiny leaves of this conifer. *Adenomera araucaria* is a predominantly diurnal species. During the breeding time from October to late January, males usually called from early afternoon to midnight. No specimens were heard at late night or at dawn. Calling activity increased after showers. Males were calling on the ground, mostly hidden between roots, stones and fallen branches. Neither eggs nor tadpoles were found but terrestrial reproduction in foam nests with non-feeding larvae is assumed (mode 22 after DUFFMAN & TRUEB, 1985), as described for most other members of the group (e.g., HIYER et al., 1990), because *Adenomera araucaria* was never observed near streams or standing water. At Pro-Mata, the new species was found living in sympatry with *Eleutherodactylus cf. guentheri*.

**Distribution** *Adenomera araucaria* occurs in the southern region of the Serra Geral, southern Brazil. At present, it is known from several municipalities in northeastern Rio

Grande do Sul, i.e., Bom Jesus, Cambará do Sul, Canela, Gramado, São Francisco de Paula and São José dos Ausentes. The municipality of São Francisco de Paula marks the southernmost distribution limit of new species, the genus and the Atlantic Rainforest Domain. Calling specimens were also heard in southern Santa Catarina, municipality of Timbé do Sul (on 4 January 1998). It is not known where the northern and western distribution limits of *A. araucaria* lie, but the species may be restricted to a small area of the southern Serra Geral. Populations from northern Santa Catarina are slightly different and probably belong to another species (see discussion). Specimens from Misiones, Argentina, have different calls (Diego Baldo, personal communication) and presumably represent the recently revalidated *Adenomera diptyx* (Boettger, 1885) (DE LA RIVA, 1996).

*Etymology* The new species is named after the Paraná pine, *Araucaria angustifolia*, in allusion to the preferred microhabitat at CPCN Pró-Mata and the difficulty of finding specimens in the spiny understory of the *Araucaria* forest. The name is used as an invariable noun in apposition.

## DISCUSSION

Differences in morphological features have traditionally been considered good evidence for allocation of specific status. With the advent of technology, the acoustic realm has undergone a boost, as it is now possible to visualize and analyze signals digitally. Advertisement calls have become as important as morphology in species determination in anurans, at times being the major discriminator between one or another population (e.g., HEYER et al., 1996). Because advertisement calls are important in species recognition, work as premating isolating barriers among sympatric species (DUELLMAN & PYLES, 1983) and tend to be rather stereotyped, workers use them for species identification in the field.

HEYER (1974) studied the relationships of the *marmoratus* species group within the family Leptodactylidae and revalidated the genus *Adenomera*. HEYER (1977) also suggested that *Adenomera marmorata* may be a composite species, although the evidence was not as clear as for *Adenomera bokermanni*. He found a broad overlap in morphological characters between different populations from southeastern Brazil and stated that advertisement call data are needed for resolving the systematics of this group (HEYER, 1984). Our comparison of calls of *Adenomera* from Rio Grande do Sul (tab. 2, fig. 3) with those from other localities in southeastern Brazil demonstrate noticeable differences. BARRIO (1965) described the call of *A. marmorata* from Paranapiacaba, state of São Paulo. At a temperature of 20°C, he described a call length of 100 ms, issued at a call repetition rate of 75 calls/min, a fundamental frequency of 2200-3200 Hz and a dominant frequency of 5200-6000 Hz. STRAUGHAN & HEYER (1976) described the call of *A. marmorata* from Tijuca, state of Rio de Janeiro (figured in HEYER, 1973). At 22°C, they reported call length to be of 100 ms and a call repetition rate of 94 calls/min, while the dominant frequency oscillated between 4500-5600 Hz, and an apparent broad fundamental below 1000 Hz, amplitude modulation apparent throughout call. HEYER et al. (1990) also reported the call of *A. marmorata* from Boracéia, São Paulo. At a temperature of 18°C, call lengths were found to vary between 40 and 70 ms in duration, issued at a rate of 0.8 to 1.4 calls/s (approximately 48-84 calls/min), with a main carrier frequency (which is also the fundamental frequency) of 4500 to 5400 Hz. Call onset was abrupt and intensity was

Table 2. Acoustic parameters for four males (a-d) of *Adenomera araucaria*. Numbers of first line are means  $\pm$  standard deviations, numbers in brackets are ranges.

Recording	(a) 8 Nov. 1995	(b) 20 Dec 1995	(c) 1 Nov. 1996	(d) 1 Nov 1996
Number of calls	4	20	11	8
Temperature ( $^{\circ}$ C)	19	26	16	16
Call length (ms)	90.0 $\pm$ 4.3 (85.5-95.7)	127.6 $\pm$ 3.1 (123.5-137.3)	129.3 $\pm$ 3.4 (125.6-136.6)	134.3 $\pm$ 2.7 (130.6-139.5)
Call rate (calls/min)	34.6	44.8	26.2	27.4
Call rise time (ms)	56.3 $\pm$ 6.0 (47.3-60.2)	57.5 $\pm$ 16.2 (41.2-81.0)	46.9 $\pm$ 11.1 (35.1-64.8)	39.1 $\pm$ 4.7 (35.6-50.4)
Dominant frequency (Hz)	5318 $\pm$ 68 (5263-5403)	5000 $\pm$ 94 (4885-5144)	4672 $\pm$ 43 (4625-4745)	4650 $\pm$ 37 (4625-4705)
Other frequencies with perceptible energy (Hz)	-	14847 $\pm$ 247 (14593-15231)	13875	13819 $\pm$ 86 (13716-13995)
Frequency modulation (Hz)	1076.7 $\pm$ 258.4 (775.3-1292.0)	1438.4 $\pm$ 218.4 (1033.6-1808.8)	822.2 $\pm$ 20.0 (861.3-947.5)	818.3 $\pm$ 46.0 (775.2-861.3)

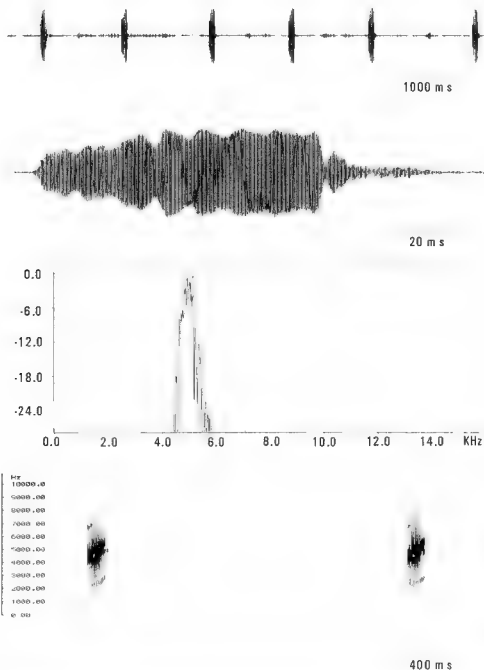


Fig 3 Oscillogram of six calls, waveform at higher resolution, power spectrum and audiospectrogram of the advertisement call of *Adenomera araucana* from Pro-Mata Rio Grande do Sul, recorded on 20 December 1995, 14:00 h, air temperature 26°C; no voucher specimen



maintained for most of the beginning of the call, declining towards end. There was upward frequency modulation within the call, and no harmonics were reported.

In addition to these reported calls, W. R. Heyer kindly made available to us recordings of *Adenomera* from an area geographically close to Rio Grande do Sul, Pirabeiraba, in Santa Catarina, and Serra da Bocaina, São Paulo (see tab 3). We have identified two distinct advertisement calls from Pirabeiraba and have analyzed recordings from two calling males ( $n = 2$ ) for each of the two call types (there is one voucher specimen for call type II and two for call type I but calls from one of the Pirabeiraba I call type vouchers were not used in analysis due to the very poor signal to noise ratio). For the population of Serra da Bocaina, we have analyzed recordings from three calling males ( $n = 3$ ). For comparative purposes, calls from Serra da Bocaina have been tentatively assigned to *Adenomera bokermanni* because: (1) the frogs were heard calling and were collected in an open formation environment, which is understood to be the preferred habitat of *A. bokermanni* (HEYER, 1984), and (2) the single voucher has slender, unexpanded toe tips (characteristic of *A. bokermanni*; HEYER, 1984) and besides a discontinuous mid-dorsal dark brown stripe running from shoulder to groin, the dorsal pattern is mostly uniform. However, nominal *A. bokermanni* has long been suspected to be a composite of more than one species (HEYER, 1977, 1984), and given that its taxonomy still remains unresolved we are conservative about allocating this population to *A. bokermanni* in a definitive way. On the other hand, the single vouchers for the two distinct call types from Pirabeiraba differ from each other as one has expanded toe tips (call type II) and a nearly rounded to rounded snout, whereas the other has slender, unexpanded toe tips (call type I) and a subelliptical to pointed snout, so we believe that there are at least three different taxa considering both localities. Unfortunately, because the number of call vouchers is limited, it is difficult to properly allocate identities to these populations, but it is important to compare the new species' call to those in geographic proximity and to note that there are more advertisement calls than available names.

With regards to reported calls, there is overlap in values of main carrier frequency for all three reported calls and the new species. There are, however, clear differences with each reported call. The new species has a much lower call rate than any of those reported in the literature, presenting no overlap with any of them, save the closeness of the Boracéia calls calling rate. There is overlap in call length with calls from Paranapiacaba and Tjuca, but calls from Boracéia are shorter than those of the new species. The Boracéia calls do not have severe amplitude modulations, which calls of the new species possess. The call from Rio de Janeiro has apparent intensity (amplitude) modulation (STRAUGHAN & HEYER, 1976), but it is difficult to tell in the case of the Paranapiacaba call as spectrogram information does not allow observation of amplitude patterns in detail. Additionally, although the number of harmonics detected will be a function of the closeness of the recording equipment to the animal and of the recording equipment per se, the new species consistently shows higher harmonics, extending to about 15 kHz.

In contrast with both call types from Pirabeiraba, calls of the new species are consistently longer. Call rate is also notably lower in the new species than that of Pirabeiraba call type I, although there is some overlap with Pirabeiraba call type II. Call rise time is generally longer in the new species than in the Pirabeiraba calls, although this parameter is subject to considerable variability. Main carrier frequency differs by about 1600-2000 Hz with Pirabeir-

Table 3 – Acoustic parameters for two distinct call types of *Adenomera* from Parabeiraba, Santa Catarina (a-d; *Adenomera* cf. *marmorata*, call type I and II) and for *Adenomera* from Serra da Bocaina, São Paulo (e-g, *Adenomera* cf. *bokermanni*). Numbers of first line are means  $\pm$  standard deviations, numbers in brackets are ranges. Question marks (?) indicate that frequency values observed may be a product of masking background noise rather than the acoustic signal being measured. *Adenomera* cf. *bokermanni* shows negative values or none at all for frequency modulation (Hz) as this modulation may be inverse (falling frequency with time rather than rising) or nonexistent.

Recording	(a) USNM 318237	(b) no voucher	(c) USNM 243740	(d) no voucher	(e) no voucher	(f) USNM 318183	(g) no voucher
Identification	<i>Adenomera</i> , call type I, Parabeiraba	<i>Adenomera</i> , call type I, Parabeiraba	<i>Adenomera</i> , call type II, Parabeiraba	<i>Adenomera</i> , call type II, Parabeiraba	<i>Adenomera</i> , Serra da Bocaina	<i>Adenomera</i> , Serra da Bocaina	<i>Adenomera</i> , Serra da Bocaina
Number of calls	10	10	10	3	10	10	10
Temperature (°C)	24.5	24.5	21.5	–	–	19-20	19-20
Call length (ms)	71.6 $\pm$ 3.7 (67.7-79.1)	88.2 $\pm$ 6.6 (80.1-98.0)	75.9 $\pm$ 3.5 (70.1-80.8)	75.9 $\pm$ 12.2 (61.9-83.9)	58.4 $\pm$ 2.3 (54.3-61.5)	52.6 $\pm$ 1.7 (51.2-55.9)	48.4 $\pm$ 4.2 (45.4-59.7)
Call rate (calls/min)	93.2	113.1	37.1	31.7	308.7	238.6	288.9
Call rise time (ms)	2.9 $\pm$ 3.6 (0.4-9.2)	7.3 $\pm$ 13.2 (0.9-43.9)	28.1 $\pm$ 25.4 (6.6-68.2)	11.9 $\pm$ 1.1 (10.6-12.7)	14.0 $\pm$ 2.2 (8.5-16.1)	16.3 $\pm$ 1.4 (13.5-18.6)	11.8 $\pm$ 2.9 (7.6-15.9)
Dominant frequency (Hz)	3038 $\pm$ 17 (3030-3070)	3034 $\pm$ 13 (3030-3070)	5343 $\pm$ 77 (5212-5443)	5285 $\pm$ 30 (5251-5311)	2284 $\pm$ 13 (2248-2288)	2213 $\pm$ 0 (2213-2213)	2327 $\pm$ 19 (2288-2367)
Other frequencies with perceptible energy (Hz)	–	–	3011 $\pm$ 189? – 10779 $\pm$ 189 (10486-10965)	7963 $\pm$ 185? – 10529 $\pm$ 61 (10462-10582)	4396 $\pm$ 155 (4177-4694) 6398 $\pm$ 87 (6273-6531)	4296 $\pm$ 691 (3808-4785)	–
Frequency modulation (Hz)	325.4 $\pm$ 134.7 (86.1-516.8)	620.2 $\pm$ 79.2 (516.8-775.2)	–	601.6 $\pm$ 0 (601.6-601.6)	(-85.9-171.9)	(-86.1-200.4)	(0-257.8)

raba call type I and there is some overlap with call type II. Calls of the new species are audibly frequency-modulated, and although there is almost no overlap with Pirabeiraba call type I in frequency modulation, this is not obvious in the case of Pirabeiraba call type II. To the ear, Pirabeiraba call type II seems to be less frequency modulated than the new species' call, although measuring this modulation was not possible due to the excessive background noise and poor signal to noise ratio. In addition, the one voucher for Pirabeiraba call type II is rather small (SVL 17.1 mm), has rounded, expanded toe tips, and a nearly rounded to rounded snout, which could fit the new species. With regards to *Adenomera* cf. *bokermanni* calls from Serra da Bocaina, these are much shorter in length than those of the new species, and call rate is several orders of magnitude higher in *A.* cf. *bokermanni* than what it is in the new species. Call rise time is also much longer in the new species' call than in *A.* cf. *bokermanni*. The main carrier frequency differs by about 2500-3000 Hz between these taxa, and frequency modulation in *A.* cf. *bokermanni* is either nonexistent, negative or minimal, whereas the new species possesses considerable frequency modulation. Overall, and with the exception of Pirabeiraba call type II, the differences in call rate, call length, main carrier, amplitude modulation and higher harmonics support that this is a distinct call distinguished from any other reported in the literature for the southeastern Brazil region.

Although the morphological characteristics of *Adenomera araucaria* revealed intraspecific variation, especially in dorsal coloration, which could make discrimination from other species of the genus difficult, the combination of several features should in most cases allow identification, i.e., small body with short arms and hindlimbs, non-expanded toe tips, whitish ventral surface of thigh without granules, inconspicuous dorsal pattern of longitudinally arranged, dark marks and development of tubercles on tarsus and sole of foot. The new species is most similar to *Adenomera marmorata*, but our examination of the holotype (NHM 16453, fig. 4a), presumably from Rio de Janeiro (BOKERMANN, 1966), revealed notable differences, e.g., larger body proportions (SVL 21.6 mm, HL 7.7 mm, HW 7.4 mm, THL 9.2 mm, TL 9.6 mm) and intensively granulated lower parts of thigh.

On the other hand, only small morphological differences were found comparing the new species with small-sized *Adenomera* from populations in northern Santa Catarina that, at present, are allocated to *A. marmorata*. Specimens from Corupá and Pirabeiraba, both localities near the border of Paraná about 50 km from each other, differ from *A. araucaria* only in subtle characteristics. Besides the toe tips being a little more expanded and the ventral surface of thighs more granular and dark mottled, the dorsal pattern is more accentuated. Our comparison with the type material of *Leptodactylus nanus* (ZSM 661/1920/1-3, fig. 4b) described from Corupá (MÜLLER, 1922) confirmed that this is a species different from *Adenomera araucaria*. However, material collected at Morro do Baú, Ilhota, about 50-100 km southeast from Corupá, differ morphologically very slightly and only gradually from *A. araucaria* and, therefore, could be conspecific with this species. The most noticeable difference is the dorsal pattern, which consists of conspicuous blotches producing a symmetrical, dark brown marbling in five of eight specimens examined. Morro do Baú lies in the crystalline coastal range of the Serra do Mar in northeastern Santa Catarina, about 200-300 km distance from the Serra Geral in Rio Grande do Sul, where the new species occurs. Both mountain ranges are geologically different and separated by the southeastern Catarinense depression. Pending further studies, including analysis of advertisement calls from Morro do Baú, this population is identified as *Adenomera* cf. *araucaria*.

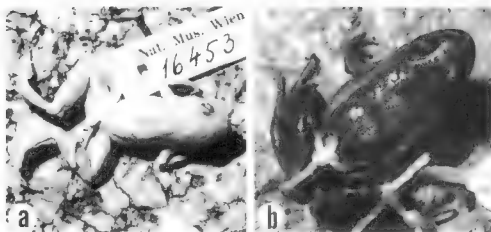


Fig 4 - (a) *Adenomera marmorata*, holotype, NHM 16453, (b) *Leptodactylus nanus*, lectotype, ZSM 661.1920.3.

Based on combined evidence from bioacoustical and morphological data, the differences between the populations of *Adenomera* in Rio Grande do Sul and other populations in southeastern Brazil (with the exception of possible conspecific specimens from Morro do Bau and call type II from Pirabeiraba) justify the allocation of specific status to the southernmost contingent of the genus in Brazil.

#### ACKNOWLEDGEMENTS

We thank Marcos Di-Bernardo, Wolf Engels and Andreas Schlüter for their assistance and for providing the opportunity for the fieldwork of AK. For critical comments on the manuscript we acknowledge W Ronald Heyer and Marcos Di-Bernardo. We are also grateful to Tatiana Miranda who kindly made the drawings and to Jochen Ketterl who collected two specimens and provided one photo. For access to material under their care we thank Marcos Di-Bernardo (MCP), Franz Tiedemann (NMW), Andreas Schlüter (SMNS), W. Ronald Heyer (USNM), Rainer Günther (ZMB), Jakob Halbermann (ZMH) and Frank Glaw (ZSM). We also thank Diego Baldo and Paulo C. A. Garcia for additional information. Field work of AK was supported by grants of the LGFG, DAAD and SHIFF (Germany). Financial support for AA was provided by operating NSERC grant #4946 to G. K. Morris.

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## APPENDIX I

## ADDITIONAL SPECIMENS EXAMINED

- Adenomera andreae* (Müller, 1923) BRAZIL: AMAZONAS, Itacoatiara SMNS 8718 1-7, PARA. PEIXEBOL. lectotype ZSM 145.1911.4, paralectotypes ZSM 145.1911.1-3 PERU Rio Yuyapichis, Panguana: SMNS 7131.
- Adenomera araucaria* sp. nov. BRAZIL: RIO GRANDE DO SUL, São Francisco de Paula, CPCN Pró-Mata: SMNS 9023
- Adenomera* cf. *araucaria* BRAZIL SANTA CATARINA, Ilhota, Morro do Baú: MCP 1345-52
- Adenomera bokermanni* (Heyer, 1973) - BRAZIL RIO DE JANEIRO, Niterói ZSM 34 1947 BRAZIL: BAHIA, Ilhéus, Centro de Pesquisas do Cacau: USNM 336245-48.
- Adenomera diptis* (Boettger, 1885) PARAGUAY paralectotype ZMB 10595
- Adenomera hylaeuctyla* (Cope, 1868) BOLIVIA Beni SMNS 9087 ECUADOR MORONA-SANTIAGO SMNS 7751, NAPOO SMNS 7762 PERU Rio Yuyapichis, Panguana. SMNS 6384, 7132, 8853 SURINAM: Caboeni creek: SMNS 8222 1-3.
- Adenomera marmorata* Steindachner, 1867 BRAZIL RIO DE JANEIRO holotype NMW 16453, SÃO PAULO, Salesópolis, Boracéia USNM 209116-20, São Sebastião island ZSM 19 1952 1-3
- Adenomera* cf. *marmorata* I (*Leptodactylus namo* Müller, 1922) BRAZIL SANTA CATARINA, Pirabeiraba USNM 243737 39, 243741-42, Rio Novo, Colonia Hansa ZMH A 01737-44, A 01746-50, lectotype ZSM 661.1920 3, paralectotypes ZSM 661.1920 1-2
- Adenomera* cf. *marmorata* II BRAZIL SANTA CATARINA, Florianópolis island MCP 1340

Corresponding editor: W. Ronald HEYER

## Régimes alimentaires de deux espèces de Bufonidae (*Bufo bufo spinosus* et *Bufo mauritanicus*) au lac Aguelmam Azegza (Maroc)

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**This study deals with the diets of two toad species (*Bufo bufo spinosus* and *Bufo mauritanicus*) through examination of the stomach contents of specimens collected in the area of the Aguelmam Azegza lake in the Moroccan Middle Atlas. The expression of results in terms of presence frequency and relative abundance suggests a similarity of the two species diets, which were both high in Coleoptera, Hymenoptera Formicidae and Dermaptera. Measures of the amplitudes and overlaps of trophic niches as well as the prey sizes exclude the existence of any kind of food competition between the two species. The diversity of the two Bufonidae diets increases during low prey availability, and decreases with prey abundance. The main preys are not significantly different between the two sexes of the same species. A comparison of several Bufonidae species around the world leads to the conclusion that their diets are primarily composed of Formicidae and Coleoptera.**

### INTRODUCTION

La connaissance de l'alimentation des Anoures dans leur milieu naturel est une étape indispensable à la compréhension de leur biologie et de leur écologie, elle peut contribuer à expliquer comment s'effectue le partage des ressources du milieu entre les espèces, à quantifier l'importance des phénomènes de compétition et à déterminer la place des Amphibiens dans les réseaux trophiques de la biocénose, sachant que ces animaux sont souvent en populations denses.

Par l'analyse quantitative des contenus stomacaux de 43 *Bufo bufo spinosus* et 48 *Bufo mauritanicus*, dans l'un des rares sites marocains où les deux espèces cohabitent, à savoir le lac Aguelmam Azegza, nous nous proposons: (1) d'étudier, pour la première fois, la composition quantitative des régimes alimentaires de ces deux espèces de Bufonidae, demeurée méconnue en dehors des données qualitatives fournies par PASTEUR et BONS (1959); (2) de comparer

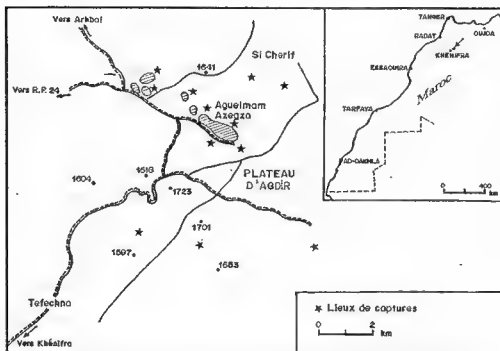


Fig. 1 Carte de situation du lac Aguelmam Azegza dans le Moyen Atlas central (Maroc)

globalement les régimes alimentaires de ces deux espèces, par le biais de l'indice  $H'$  de Shannon en tant que mesure l'amplitude de la niche trophique (BARBAULT, 1974) et de l'indice  $R$  de recouvrement des niches trophiques (PIANKA, 1973); (3) d'analyser le spectre de tailles et la composition spécifique des proies consommées pour vérifier la présence ou l'absence d'une compétition alimentaire entre ces deux espèces. Soulevant le problème de la place des Amphibiens dans la chaîne trophique, la présente étude s'inscrit dans le cadre d'un travail plus général consacré à l'étude de la place des Amphibiens dans les réseaux trophiques des écosystèmes lacustres du Moyen Atlas.

#### TERRAIN D'ÉTUDE ET MÉTHODES

Le Moyen-Atlas central, massif bien arrosé aux terrains calcaires et dolomitiques parsemés de dolines karstiques, offre une quinzaine de lacs naturels permanents (fig. 1). Le lac Aguelmam Azegza, situé à une altitude de 1470 m avec une latitude de  $32^{\circ}58'50''$ - $32^{\circ}58'15''$ N et une longitude de  $05^{\circ}26'15''$ - $05^{\circ}27'30''$ W, occupe une profonde dépression entourée de reliefs calcaires couverts par une belle forêt de chêne vert et de cèdre. Sa superficie est d'environ 50 ha et sa profondeur maximale est de 26 m. Le bioclimat de la région est du type sub-humide à humide. Les précipitations moyennes sont de 1150 mm/an, en grande partie

sous forme de neige qui peut parfois persister sur les hauts versants jusqu'au mois de mars. La température mensuelle moyenne varie entre  $-2^{\circ}\text{C}$  et  $31^{\circ}\text{C}$ . Durant les mois de décembre à février, elle n'excède pas les  $9^{\circ}\text{C}$ , alors qu'en automne elle se situe entre  $2$  et  $22^{\circ}\text{C}$ . Le lac Aguelmam Azegza est fréquenté par la plupart des Anoures de la région pendant leurs périodes de reproduction, les espèces les plus abondantes étant *Rana saharica*, *Hyla meridionalis*, *Bufo bufo spinosus* et *Bufo mauritanicus*. La présence de *Discoglossus pictus* est également notée. L'activité des deux Bufonidés commence après une longue période d'hibernation entre novembre et février, et s'étend sur la période mars-octobre.

L'analyse porte sur 91 Crapauds adultes (43 *Bufo bufo spinosus* et 48 *Bufo mauritanicus*) récoltés de nuit entre 20 h et 1 h, de février 1988 jusqu'en mars 1990, avec des échantillonnages mensuels. La plupart des spécimens ont été récoltés dans un rayon de 6 km autour du lac. Ils appartiennent au même peuplement, tous les individus des deux espèces fréquentant le lac pendant leur période de reproduction. Aussitôt prélevés, tous les Crapauds ont été fixés, cavité viscérale ouverte, dans l'alcool à  $70^{\circ}$  mélangé au formol à  $4^{\circ}$ , afin d'arrêter rapidement la digestion post-mortem (GRANVAL, 1987). Les contenus stomacaux ont été extraits dans une solution d'alcool, puis examinés sous une loupe binoculaire équipée d'un micromètre permettant la mesure de la taille des proies. Le statut taxinomique des proies a été déterminé jusqu'à la famille. La détermination du genre, voire de l'espèce, dépendait du stade de digestion des proies. Une identification préliminaire a été faite grâce aux différentes clés qui existent à l'Institut Scientifique de Rabat; elle a souvent été suivie par une vérification à l'aide des collections de ce même institut.

Les résultats ont été exprimés de différentes façons complémentaires, afin de mieux assurer leur interprétation.

(1) L'abondance relative des catégories de proies, calculée à l'aide de la formule  $P_i = (A_i/n) \times 100$ , où  $A_i$  est le nombre d'individus de la catégorie de proies considérée et  $n$  est le nombre total d'animaux dénombrés dans le tube digestif. Cette estimation attribue la même importance à des proies de valeur énergétique et de taille plus ou moins inégales (LESCURE, 1973).

(2) Le degré de présence, défini par la formule  $C_i = (S_i/S) \times 100$ , où  $S_i$  est le nombre d'estomacs contenant la catégorie de proie  $i$  et  $S$  est le nombre total d'estomacs examinés. Ce paramètre constitue, du point de vue éthologique, une mesure de la préférence du Batracien étudié pour un type de proie  $i$  (LESCURE, 1971).

(3) L'amplitude de la niche trophique, mesurée par l'indice de Shannon (BARBAULT, 1974)  $H' = -\sum P_i \log_e P_i$ , où  $P_i$  est l'abondance relative de la catégorie de proie  $i$ .

(4) Le coefficient de vacuité  $V = (E_1/E) \times 100$  où  $E_1$  est le nombre total d'estomacs vides et  $E$  est le nombre d'estomacs examinés.

(5) L'indice de recouvrement des niches trophiques  $R$  (PIANKA, 1973; FORGI & BARBAULT, 1978):

$$R = \frac{\sum P_{ij} \times P_{ik}}{\sqrt{\sum P_{ij}^2 \times P_{ik}^2}}$$



où  $P_{ij}$  et  $P_{ik}$  représentent l'abondance relative d'une catégorie de proie  $i$  dans le régime alimentaire de deux espèces prédatrices  $j$  et  $k$ . Cet indice de similitude permet de comparer des spectres d'utilisation de ressources alimentaires, sans qu'il soit un véritable indice de compétition (BARBAULT, 1981).

Le test de Chi-deux ( $\chi^2$ ) sur les abondances relatives des proies a été employé afin d'établir s'il existe une différence significative entre le régime alimentaire des mâles et des femelles (SOLANO, 1983).

## RÉSULTATS ET DISCUSSION

### L'ALIMENTATION DE *BUFO BUFO SPINOSUS*

Il est important de signaler que cette espèce ne cherche pas sa nourriture durant la période de reproduction où le coefficient de vacuité  $V$  est de 100 %. Plusieurs observations dans ce sens ont été publiées (LESCURL, 1965, CHRISTIAN, 1982). Cette constatation ne corrobore pas les prévisions théoriques des régimes optimaux qui envisagent un apport énergétique élevé durant la période de reproduction (MAC ARTHUR & PIANKA, 1966). Le comportement agonistique, comme les autres activités associées à la reproduction, impliquent des dépenses d'énergie élevées (SCHONER, 1971). *Bufo bufo spinosus*, à l'issue d'une longue période d'hibernation à jeun, semble investir presque tout son temps dans des activités reproductrices. Il possède la plus courte et précoce période de reproduction connue chez les Anoures de la région d'étude (mi-février - fin mars, CHILLASSE, 1990).

L'alimentation de *Bufo bufo spinosus* est composée de 13 catégories de proies, représentées dans des proportions très différentes, les Coléoptères, les Dermoptères et les Formicidae représentant 90 % de l'effectif total (tab. 1, fig. 2). Parmi les Coléoptères, les Curculionidae, actifs la nuit, l'emportent de loin sur les autres familles ( $P_i = 15\%$ ,  $C_i = 58\%$ ); les genres les plus représentés sont *Otiorhynchus*, *Tennorhinus* et *Thylactes*. Les Tenebrionidae et les Pterostichidae viennent en seconde position, suivis par les Scarabaeidae et les Harpalidae. Les autres familles sont peu représentées dans l'alimentation de cette espèce. Elles sont, pour la plupart, diurnes ou crépusculaires et vivent dans des endroits peu accessibles aux Crapauds. Les Dermoptères sont représentés par deux espèces, *Forficula auricularia* (Forficulidae) et *Labiduria riparia* (Labiduridae), avec une abondance relative  $P_i$  de 35 % et un degré de présence  $C_i$  de 46 %. Les Hyménoptères (Formicidae) se trouvent dans presque 40 % des contenus stomacaux examinés, avec une abondance relative de 22 %. Les Arachnides, les Myriapodes et les larves d'Insectes sont moyennement représentés. Notons la présence d'une petite Couleuvre (*Natrix maura*) dans l'estomac d'un Crapaud commun. Des observations similaires ont été rapportées par LOVERIDGE (1936).

L'étude comparative de la composition des contenus stomacaux des deux sexes de *Bufo bufo spinosus* montre que ceux-ci utilisent les mêmes ressources alimentaires durant toute leur période d'activité. Il n'y a pas de différence significative entre les profils d'abondance des proies chez les deux sexes ( $\chi^2$ ,  $df = 12$ ,  $P > 0.05$ ).

Tableau 1 – Abondance relative et degré de présence des différentes catégories de proies dans les contenus stomacaux de *Bufo bufo spinosus* et *Bufo mauritanicus* dans la région du lac Aguelmaïm Azegza (Maroc).

Catégories de proies	Abondance relative (Pi)		Degré de présence (Ci)	
	<i>B. bufo spinosus</i>	<i>B. mauritanicus</i>	<i>B. bufo spinosus</i>	<i>B. mauritanicus</i>
(1) Coléoptères (Col)	31	50	62	93
Curculionidae	15	17	58	51
Tenebrionidae	5	5	41	62
Pterostichidae	5	6	50	44
Scarabaeidae	4	8	14	25
Harpalidae	1	12	14	60
Staphylinidae	0.3	1	6	14
Chrysomidae	0.3	0.05	2	2
Helophoridae	0.1	0	2	0
Elateridae	0.1	0.05	2	2
Cerambycidae	0.05	0	2	0
Histeridae	0	0.2	0	7
Lebidae	0	0.1	0	4
Meloidae	0	0.05	0	2
Melyridae	0	0.05	0	2
(2) Diptères (Dipt)	0.3	1.5	8	16
Tipulidae	0.2	1	6	11
Muscidae	0	0.1	0	4
Tachinidae	0.05	0	2	0
(3) Hyménoptères (Hym)	23	18	39	62
Formicidae	22	16	35	62
Myrmecidae	1	2	6	23
Ichneumonidae	0.1	0.2	4	2
Pompilidae	0	0.1	0	2
(4) Hémiptères (Hém)	1	1.5	14	21
Pentatomidae	0.3	1	8	11
Carpidae	0.05	0.05	2	2
Lygaeidae	0.05	0.04	2	2
Nabidae	0.05	0.05	2	2
(5) Dermaptères (Der)	35	21	46	46
Forficulidae	28	19	42	39
Labiduridae	7	2	23	14
(6) Orthoptères (Ort)	0.05	0.3	2	9
Gryllidae	0.05	0	2	0
Acrididae	0	0.3	0	9
(7) Lépidoptères (Lép)	0.2	1	6	25
Noctuidae	0.2	1	6	25
(8) Larves d'insectes (Lar)	2	2	29	37
L. Coléoptères	1	1	21	23
L. Lépidoptères	0.7	1	21	11
L. Diptères	0.1	0.05	4	2
L. Hémiptères	0	0.05	0	2
L. Odonates	0.05	0	2	0
(9) Arachnides (Ara)	4	2	44	37
Thomisidae	0.5	0.2	4	9
Agelenidae	0.4	0	10	0
Dysderidae	0.3	0.05	8	2
Lycosidae	0.3	1	10	9
Linyphiidae	0.2	0	2	0
Gnaphosidae	0.1	0.1	2	4
Sparassidae	0.05	0	2	0
Phalangidae	1	1	23	21
Buthidae	0	0.05	0	2
(10) Isopodes (Iso)	3	3	48	51
Armadillidae	2	1	31	16
Porcellionidae	2	2	29	35
(11) Mymapodes (Myr)	0.3	1	12	16
Lithobiidae	0.1	0.2	5	4
Scolopendridae	0.05	0.05	2	2
Lulidae	0.2	1	6	14
(12) Gastéropodes (Gau)	0.1	0	2	0
Physidae	0.1	0	2	0
(13) Reptiles (Rep)	0.05	0	2	0
Colubridae	0.05	0	2	0

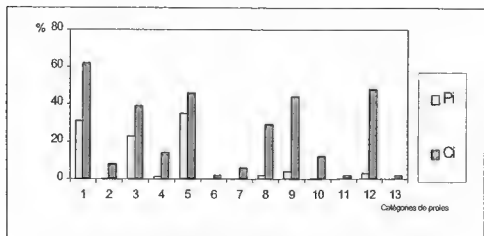


Fig. 2. - Composition des contenus stomacaux de *Bufo bufo spinosus*

#### L'ALIMENTATION DE *BUFO MAURITANICUS*

Pendant la période de reproduction de cette espèce (début mars - fin mai), le coefficient de vacuité s'est révélé très faible ( $V = 7\%$ ), sans être nul comme chez l'espèce précédente.

Le spectre alimentaire est formé de 11 catégories de proies avec toujours une dominance des Coléoptères, des Hyménoptères (Formicidae) et des Dermaptères qui totalisent respectivement des abondances relatives de 50 %, 21 % et 18 % (tab. 1, fig. 3). Parmi les Coléoptères, les Curculionidae (*Otorhynchus*, *Brachyderes* et *Othylactes*) et les Harpalidae (*Harpalus*, *Acinopus* et *Ditomis*) l'emportent de loin sur toutes les autres familles. Mis à part les Lépidoptères nocturnes (Noctuidae) activement chassés, les Insectes volants (Diptères, Hyménoptères autres que les Formicidae, Héteroptères, Orthoptères) sont négligeables dans l'alimentation de l'espèce, leurs abondances relatives ne dépassant pas le seuil de 1 %.

La comparaison entre les régimes des mâles et des femelles montre, pour les trois groupes les plus consommés, des proportions similaires chez les deux sexes. Les autres groupes, dont la contribution dans l'alimentation de ce Crapaud est très faible, présentent de légères différences d'abondance : les Isopodes plus consommés par les mâles, alors que les femelles ingèrent plus d'Hétéroptères. Il n'y a pas de différence significative entre mâles et femelles si l'on considère la totalité des proies ( $\chi^2$ ,  $df = 10$ ,  $P > 0.05$ ).

#### MESURE DE L'AMPLITUDE DES NICHES TROPHIQUES

En dehors de la période de reproduction de *Bufo bufo spinosus*, marquée par un coefficient de vacuité très élevé, les spectres alimentaires des deux espèces sont très diversifiés. Étant donnée la ressemblance très marquée entre les régimes alimentaires des deux espèces, nous avons eu recours à plusieurs tests statistiques pour confirmer ou rejeter l'hypothèse d'une compétition alimentaire.

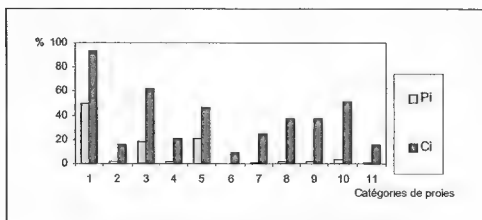


Fig. 3. – Composition des contenus stomacaux de *Bufo mauritanicus*.

L'indice de Shannon  $H'$ , utilisé comme mesure de l'amplitude de la niche trophique pour chaque récolte mensuelle (fig. 4), montre chez *Bufo bufo spinosus* des valeurs relativement faibles durant les mois de mai, juin et juillet, période pendant laquelle les ressources alimentaires sont très abondantes, mais constituées essentiellement de Coléoptères et de Dermaptères. Chez *Bufo mauritanicus*, cet indice enregistre de faibles variations durant toute sa période d'activité, les Coléoptères, les Dermaptères et les Formicidae étant toujours dominants.

La contraction estivale, période de pleine abondance, des régimes alimentaires des deux espèces semble, a priori, vérifier une des prévisions de la théorie des régimes optimaux (MAC ARTHUR & PIANKA, 1966) qui envisage que, lorsque la densité des proies est élevée, le prédateur se concentre sur celles qui présentent la meilleure valeur énergétique, mais qu'au fur et à mesure que les proies se font rares, la diversité du régime augmente. Selon SCHLUTER (1981), les prévisions de la théorie des régimes optimaux ne sont pas valables pour prédire le comportement des prédateurs dans un écosystème ou les réserves et la disponibilité de la nourriture sont diversifiées et abondantes. Plusieurs facteurs entrent en jeu dans le choix des proies (disponibilité, comportement de prédation, etc.) L'hypothèse du choix d'un aliment selon sa seule valeur énergétique est peu réaliste et s'applique mal à des communautés naturelles.

#### RECouvreMENT DES NICHES TROPHIQUES

Le recouvrement des niches trophiques des deux espèces est assez modéré ( $R = 0,126$ ) durant la période de faible abondance des proies (février, mars et avril), mais il devient élevé ( $R = 0,36$ ) pendant la période des pics d'abondance des proies (mai, juin et juillet). Les valeurs faibles de  $R$  enregistrées, au moment le plus critique dans la recherche de la nourriture, prouvent que la compétition entre les deux espèces est très faible. Les chevauchements des niches trophiques, pendant la période de pleine abondance des proies, sont plutôt déterminés par la grande disponibilité de la nourriture que par une compétition. Rappelons, à ce propos,

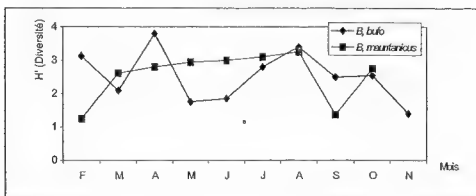


Fig. 4. - Variation de l'amplitude de la niche trophique de *Bufo bufo spinosus* et *Bufo mauritanicus*.

que cet indice de similitude permet de comparer des spectres d'utilisation de ressources alimentaires, sans qu'il soit un véritable indice de compétition (BARBAULT, 1981).

#### COMPOSITION SPÉCIFIQUE DES RÉGIMES ALIMENTAIRES

Le chevauchement des niches trophiques des deux Bufonidae étudiés, durant certaines périodes de leur activité, n'implique pas l'identité parfaite de leurs régimes alimentaires. En effet, une différence a été notée au niveau des genres de Coléoptères consommés. C'est ainsi que parmi les Curculionidae, entre autres, on a trouvé chez *B. bufo spinosus* surtout les genres *Otiiorhynchus*, *Temnorhynchus* et *Thylacites*, et chez *B. mauritanicus* les genres *Otiiorhynchus*, *Brachyderes* et *Othylacites*. La même constatation s'applique aux genres d'Harpalidae. *B. bufo spinosus* consomme surtout des *Acromopus* et des *Stenolophus*, tandis que *B. mauritanicus* se nourrit de *Dutomus*, toutefois, le genre *Harpalus*, très abondant, se trouve dans les contenus stomacaux des deux Bufonidae. La dominance des Coleoptères et des Formicidae dans le régime de ces deux Crapauds concorde, en grande partie, avec les conclusions de nombreux auteurs qui ont travaillé sur le régime alimentaire des Bufonidae.

En effet, une comparaison des régimes alimentaires des Bufonidae étudiés dans diverses régions du monde, en particulier au niveau des catégories de proies les plus consommées et dominantes (Coléoptères et Formicidae), permet de conclure que les Coleoptères et les Formicidae dominent nettement et représentent toujours plus de la moitié des proies ingérées (tab. 2). L'originalité de nos résultats réside dans la dominance des Dermapteres en plus de ces deux groupes d'Insectes (CHILLASSE, 1990). Chez les Crapauds d'Afrique équatoriale et de Malaisie, les Formicidae prédominent dans la nourriture. Ceci a été vérifié particulièrement chez *Bufo funereus* (INGER & MARX, 1961) et *Bufo regularis* (PAULLIAN & VILARDEBO, 1946; CHAPMAN & CHAPMAN, 1958; INGER & MARX, 1961; LESCURE, 1971; BARBAULT, 1974). Dans les régions tempérées européennes, les Formicidae et les Coleoptères sont d'égale importance

Tableau 2. - Abondance relative et degré de présence des Coléoptères et des Fourmis dans les régimes alimentaires de différentes espèces de Bufonidae.

Espèces	Pays, régions	Fourmis		Coléoptères		Références
		Pt (%)	Ci (%)	Pt (%)	Ci (%)	
<i>Bufo asper</i>	Malaisie:					BERRY, 1970
	- (Forêt hygropile)	33	100	0.5	38	
	- Cavernea	54	65	3	62	
<i>Bufo melanostictus</i>	Malaisie:					BERRY & BULLOCK, 1962
	- Savane et forêts	45	91	3	44	
	- Champs	19	57	8	40	
<i>Bufo regularis</i>	Basse Côte d'Ivoire	87	100	12	73	PAULLIAN & VILARDEDO, 1946
	Tanzanie	-	42	-	76	CHAPMAN & CHAPMAN, 1958
	Sénégal	55	88	12	71	LESCURE, 1971
	Côte-d'Ivoire	93	91	2	46	BARBAULT, 1974
<i>Bufo fuvareus</i>	Congo	-	87	-	68	INGER & MARX, 1961
<i>Bufo melanopleura</i>	Congo	-	100	-	77	INGER & MARX, 1961
<i>Bufo usberanus</i>	Congo	-	95	-	47	INGER & MARX, 1961
<i>Bufo pentoni</i>	Sahel Sénégalais	53	89	29	89	FORGE & BARBAULT, 1978
<i>Bufo woodhousei</i>	U.S.A.	35	50	49	95	BUSH & MENHINK, 1962
<i>Bufo calamita</i>	Espagne	25	-	53	-	LIZANA et al., 1986
<i>Bufo bufo</i>	Espagne	88.5	-	6.5	-	LIZANA et al., 1986
	France	63	84	14.3	96	LESCURE, 1965
	France	56	-	23	-	DUPONT, 1962
	Angleterre	41.6	-	16	-	COTT, 1932
<i>Bufo bufo spinosus</i>	Maroc	23	55	37	80	CHILLASSE, 1990
<i>Bufo mauritanicus</i>	Maroc	16	62	50	93	CHILLASSE, 1990

dans la nourriture des Bufonidae (DUPONT, 1962, LESCURE, 1965, LIZANA et al., 1986). En revanche, les Coléoptères supplantent les Formicidae chez les Crapauds du Maroc (CHILLASSE, 1990) et ceux d'Amérique (BUSH & MENHINK, 1962).

## CONCLUSION

Les deux espèces étudiées sont qualifiées de polyphages. Bien qu'en des proportions différentes, elles puisent leur nourriture parmi une faune d'Arthropodes abondante dans le milieu d'étude. En termes de fréquence d'occurrence et d'abondance relative, les Coléoptères, les Formicidae et les Dermapteres dominent nettement leur alimentation. Au contraire, les Arachnides, les Isopodes, les Myriapodes et les Gasteropodes comptent peu dans l'alimentation.

Les deux sexes, pour chaque espèce, paraissent avoir des régimes similaires, aucune ségrégation sexuelle pour la collecte de la nourriture n'a été signalée.

Les ressources trophiques diffèrent entre les deux espèces au regard de plusieurs facteurs: (1) les habitats fréquents sont différents: *Bufo bufo spinosus* est plus inféodé au milieu forestier que *Bufo mauritanicus* qui préfère les milieux dégagés, clairières et prairies humides, (2) la composition spécifique des proies consommées diffère. Ces facteurs assurent une

separation écologique et une cohabitation de ces deux prédateurs. Par ailleurs, la grande quantité et la variété des proies qu'ils consomment soulignent l'importance de ces Bufonidae dans les réseaux trophiques de cet écosystème péri-lacustre.

## RÉSUMÉ

L'étude concerne le régime alimentaire de deux espèces d'Amphibiens Anoures de la famille des Bufonidae (*Bufo bufo sponosus* et *Bufo mauritanicus*) dans la région du lac Aguelmam Azegza situé dans le Moyen Atlas Marocain. L'expression des résultats en termes de fréquence d'occurrence des proies (degré de présence) et d'abondance relative suggère une ressemblance des régimes alimentaires des deux espèces, avec une dominance des Coléoptères, des Hyménoptères Formicidae et des Dermaptères. Les mesures de l'amplitude et des chevauchements des niches trophiques ainsi que la taille des proies excluent la présence de toute forme de compétition alimentaire entre ces deux espèces. La diversité des régimes alimentaires des deux Bufonidae s'accroît en période de faible disponibilité pour devenir très faible en période des pics d'abondance des proies. Les deux sexes de la même espèce présentent des régimes similaires, aucune différence significative n'a été enregistrée au niveau des proies essentielles. Une comparaison des régimes de plusieurs Bufonidae étudiés dans diverses régions du monde permet de conclure que les Coléoptères et les Formicidae dominent leurs alimentations.

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Corresponding editor: Thierry Lodé



## Results of the first batrachian survey in Europe using road call counts

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Within the last 20 years, there have been extensive efforts to monitor populations of calling amphibians, especially in North America. One such initiative involves use of volunteers in conducting road call counts. To date, no attempt has been made to test the efficacy of this technique in Europe. This paper summarizes research involving road call counts in the Biharugra Landscape Protected Area, Körös-Maros National Park, Hungary. Seven of Hungary's 12 anuran species were identified in the study area using this method and an additional 3 species were detected by complementary visual encounter surveys. Limitations, including variations in species calling radii, extraneous noise and program resource requirements should be considered when designing similar volunteer-based road call count protocols for other regions. However, this method should be of value in many areas in Hungary and Central Europe, due to its low cost, accessibility of volunteers, and value in accurately detecting most anuran species (including *Bombina bombina* and *Hyla arborea* – both IUCN Red Data Book species).

### INTRODUCTION

Widespread declines of amphibian populations, often without an apparent proximate cause (BLAUSTEIN & WAKE, 1990; PHILLIPS, 1990; WAKE et al., 1991; GRIFFITHS & BEEBEE, 1992), have initiated a critical global review of the status of amphibian species (VIAL & SAYLOR, 1993). Complicating the understanding of this decline are the naturally high fluctuations of many amphibian populations (PECHMANN & WILBUR, 1994). Amphibians may also display metapopulation dynamics, with decreases in some local populations coinciding with increases in others (SJOGREN, 1991). Moreover, amphibians have been recognized as potential indicators of environmental change (VITT et al., 1990; STEBBINS & COHEN, 1995; BOWERS et al., 1998), an additional factor driving inventory and monitoring efforts. To assess the status of amphibian populations, distribution patterns and population characteristics need to be examined. However, assessments are difficult because few comparable data sets and long-term studies exist (BLAUSTEIN, 1994; REED & BLAUSTEIN, 1995). The need to establish long-term inventories and monitoring has been emphasized, both in Hungary and elsewhere (PECHMANN & WILBUR, 1994; KORSÓS, 1997).

A number of species-specific considerations may affect detection of amphibians and effective use of various survey methods. Breeding season and diurnal patterns may vary with species and site (PÉCHY & HARASZTHY, 1997; BRIDGES & DORCAS, 2000). Some populations, species, or life history stages may be easily observed, while others, being more rare, cryptic or fossorial, may require refined experience or trapping techniques. In addition, many biologists believe that a few successful populations can contribute most of the reproductive output for all populations in a local area (SOULÉ, 1987; PULLIAM, 1988; SJÖGREN, 1991). In these situations, surveys based on distinctive courtship vocalizations may prove to be the best possible method for detecting anuran species.

The Declining Amphibian Populations Task Force (DAPTF), now affiliated to IUCN, was established to develop programs in participating countries (WAKE et al., 1991; VIAL, 1991; HALLIDAY & HEYER, 1997). The road call count (RCC) method has been a frequently chosen monitoring technique in North America because of its relative ease for volunteers, and many Canadian provinces and USA states have used similar monitoring methods (see LANNOO, 1998). However, Hungarian data are less comprehensive, and although monitoring programs do exist in Europe (GASC et al., 1997), the RCC methodology has never been tested here.

Of the 74 amphibian species in Europe, 17 occur in Hungary, including 12 anurans (NÖLLERT & NÖLLERT, 1992). Hungary was one of the first European nations to enact legislation protecting its wildlife, with its herpetofauna protected as early as 1947 (CORBETT, 1989). However, like the rest of Europe, amphibians in Hungary have not received a proportionate degree of conservation action or resource allocation compared to animal groups such as birds and mammals (BAKÓ et al., 1992; PUKY, 2000). The IUCN (ANONYMOUS, 1993) recognizes that this lack of knowledge is a threat to the wetland diversity of the region.

Urban and agricultural development have had profound impacts on amphibian habitats in Hungary, including the loss and alteration of lentic habitats and their historical hydrological regimes. Vigorous programs of wetland drainage and channelization of the Tisza and Körös rivers (in the study region) in the mid-1800s, primarily for conversion to arable land, resulted in loss of many ox bow lakes (MAROSI & SZILÁRD, 1969). Lentic habitats provided by river side channels, wooded flood plain areas and off-channel sloughs and swales have been largely eliminated. For those temporary ponds which have remained or have been artificially excavated in the Tisza River basin, eutrophication is a major problem since the traditional yearly inundations have ceased (DENISOV et al., 1997). Thus, it is clear that without protective intervention, the risk of threats to amphibian populations due to, inter alia, habitat loss and deterioration, will likely increase. Although there have been some attempts to describe amphibian species and distributions in the region (MARIÁN, 1963; GUBÁNYI, 1992), there has been little effort to develop a comprehensive list of amphibian species in the Körös-Maros National Park (KMNP).

Currently, amphibian monitoring is a new focus of attention in Hungary, particularly with its obligations in planned accession to the European Union. Until this study, no wide scale, long term investigations have been conducted, yet there is a growing realization that especially with limited resources, monitoring populations must employ a number of techniques, including those that involve volunteers (KORSÓS, 1997). The goal of this study was to

help standardize methods of amphibian monitoring in Hungary and to conduct an investigation on the applicability of volunteer-based RCCs in Europe, given their widespread use in North America.

## STUDY AREA

The 52 000 ha KMNP in east Hungary is a mosaic of large and small habitats. It lies within the Great Hungarian Plain in one of the warmest (10-10 °C annual mean temperature) and driest (550-600 mm annual precipitation) regions of Hungary (ANONYMOUS, 1993). Protecting the rare flora and fauna in this region is of national importance and deserves special attention (BIRÓ, 1996). The study area, located in the 9645 ha Biharugra Landscape Protected Area, includes over 1900 ha of fishponds (fig. 1), Hungary's second largest artificial lake complex. Surrounded by vast reed beds, the ponds provide critical breeding habitats for a large number of protected bird species and for mammals, fish, reptiles and amphibians (ANONYMOUS, 1997). Owing to its rich, diverse habitat and landscape features, the ponds and surrounding marshes gained international importance and were declared a Ramsar Convention on Wetlands of International Importance Especially as Waterfowl Habitat site in 1997

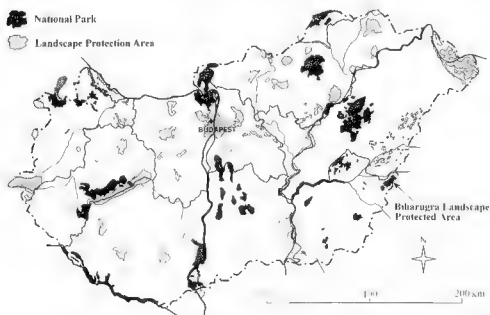


Fig. 1. Location of the study area in Biharugra Landscape Protected Area of Körös-Maros National Park, Hungary

## MATERIALS AND METHODS

A RCC route between Biharugra and Zsadány was selected for monitoring because of the area's unique diversity of amphibian habitats including vernal pools, drainage canals, fish ponds, wooded swamps and marshes. These habitats exist among agricultural land that focuses on wheat production and livestock grazing. Ten RCC stations were established running in a south and westerly direction from Biharugra (fig. 2).

The methodology in this research was based on the protocol developed by DAPTF Canada for Ontario, i.e., of the North American Amphibian Monitoring Program (NAAMP) specified in GARTSHORE *et al.* (1997). In Ontario, the route is chosen by the volunteer (thus, non-random) and ideally consists of a straight, quiet road with 10 stations 0.8 km apart, regardless of proximity to wetlands. Volunteers are requested to conduct three surveys over the anuran breeding season, corresponding with optimum weather conditions and calling periods for local species. Surveys are conducted between 30 min after sunset and midnight, with participants listening at each station for a period of 3 min, recording all anuran species heard according to the Wisconsin Index: (0) none heard; (1) individuals can be counted, no overlapping calls; (2) calls overlapping, but distinguishable; (3) full chorus, calls continuous and overlapping. Supplementary information including time, air and water temperatures, wind speed, and land use are also recorded.

In this study, I carried out RCCs between 6 March and 29 April 1998, using the Ontario methodology with the following modifications: (1) European species were identified according to the audio reproductions of anuran calls by ORSZÁGH (1982) and ALSCHER *et al.* (1998); (2) RCCs were conducted on 19 evenings instead of the suggested three to attempt to detect calling intensities of each species over the breeding season; (3) if present, extraneous noise was described for each location; (4) a 60 s, instead of 30 s, waiting period was used after alighting from the vehicle or following traffic noise before beginning or resuming the survey; (5) air temperatures were taken at the start and finish of each survey, with the mean value presented (fig. 3). As MOSSMAN *et al.* (1998) noted, measuring water temperature was time-consuming for volunteers. In this study, it was taken once per survey at station 6 to serve as a general indicator only.

To determine how well the RCC detects species presence, visual encounter surveys (VES) were conducted on two evenings (15 and 25 April) at four shallow ponds (fig. 2) located near the RCC stations (pond A, 450 m from station 2, 0.25 ha; pond B, 100 m from station 10, 0.56 ha; pond C, 60 m from station 10, 0.001 ha; pond D, 1100 m from station 10, 0.8 ha). These ponds were selected due to their easy access and because anurans were calling from these locations during the RCCs. During these evenings, RCCs were conducted, recording species heard directly from ponds A (station 2), B, C and D (station 10). Immediately following these RCCs, thorough VES were conducted around the perimeter of the ponds as recommended by THOMAS *et al.* (1997). A survey was first conducted around the shoreline examining the pond littoral zone, followed by a second walk about 1.5 m from the shoreline, encompassing a 3 m wide sweep of the riparian zone. During these walks, stops were made every 2-3 m to scan ahead for any anurans. This method also allowed detection of species calling underwater or among thick vegetation. Only adults were recorded.

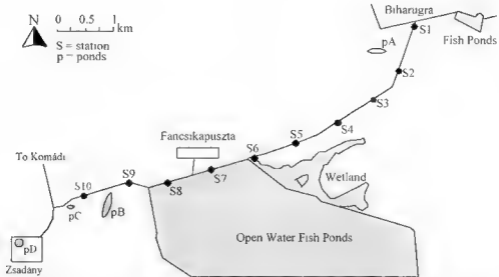


Fig 2 - Road call count route, including stations and study pond locations. Geographical coordinates of stations and study ponds (ANONYMOUS, 1995a-b) (S1) 21°35'32"E, 46°57'45"N, (S2) 21°35'22"E, 46°57'20"N; (S3) 21°35'02"E, 46°57'02"N, (S4) 21°34'30"E, 46°56'48"N, (S5) 21°33'54"E, 46°56'36"N, (S6) 21°33'19"E, 46°56'27"N, (S7) 21°32'42"E, 46°56'19"N, (S8) 21°32'05"E, 46°56'12"N, (S9) 21°31'32"E, 46°56'12"N, (S10) 21°30'55"E, 46°56'03"N, (pA) 21°35'11"E, 46°57'31"N, (pB) 21°31'00"E, 46°56'03"N, (pC) 21°30'50"E, 46°56'04"N; (pD) 21°29'45"E, 46°55'31"N

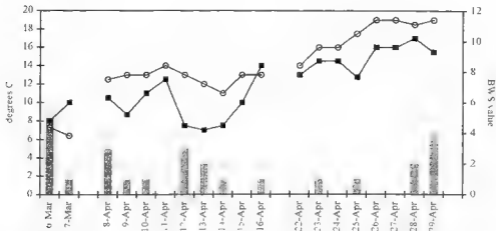


Fig 3 Beaufort Wind Scale values (shaded bars) and mean air (darkened squares) and water temperatures (open circles) during RCC.

## RESULTS

### ROAD CALL COUNTS

Seven of Hungary's 12 anuran species were detected at RCC stations along the Biharugra route. *Bombina bombina*, *Bufo viridis*, *Hyla arborea* and *Rana esculenta* were each recorded at all 10 stations (tab. 1). These four species were also heard on more evenings than any other species. Number of species recorded at each station ranged from 4 to 7.

### ENVIRONMENTAL PARAMETERS

Maximum Beaufort Wind Scale (BWS) values, and mean air and water temperatures for each RCC are shown in fig. 3. BWS values ranged from 0 to 5 (mean 1.4). Although air temperature ranged from 7.0 to 17.0°C and water temperature from 6.5 to 19.0°C at station 6 during the surveys, the onset of anuran calling was characterized when air and water temperatures first reached 10.5 and 12.5°C, respectively. However, anurans continued calling even when temperatures dropped below these values during the research period (e.g., 13-14 April).

### DURATION OF RCC

Mean time taken to conduct an individual RCC, including the observation period at each of the 10 stations, allowing time for driving and additional waiting periods in lieu of traffic noise, etc., was 75 min ( $s$  18.8; range 50-110).

### VISUAL ENCOUNTER SURVEYS

Adults of 10 of Hungary's 12 anuran species were detected during the VES (tab. 2). In some cases, due to calling underwater or among thick vegetation, individuals were heard during the VES but not seen (i.e., *Pelobates fuscus* at ponds A and C, *Bufo bufo* and *R. esculenta* at pond B).

## DISCUSSION

My road call counts revealed that anuran species richness in the Biharugra Landscape Protected Area is almost two-fold greater than the KMNP Management Plan indicated. This richness includes *Hyla arborea* and *Bombina bombina* – both IUCN International Red Data Book species (BAILLI & GROOMBRIDGE, 1996). In itself, this would be a sufficient reason to encourage the use of RCCs in other areas of the KMNP, as well as other national parks. Minimally, the use of RCCs in Hungary might be used to locate breeding amphibian populations to target for more intensive survey strategies, thereby limiting the number of sites that need to be surveyed. Indeed, the Zsadány pond (pond D) was located in this fashion (i.e., anurans calling in this pond, including the two IUCN listed species above, were heard from RCC station 10 – over one kilometre away).

Table 1. – Percentage of evenings anuran species heard at RCC stations during research period † IUCN Red Data Book (BAILLIE & GROOMBRIDGE, 1996). \* Bern Convention Appendix II (ANONYMOUS, 1994). ‡ Diminishing over European Range.

Species	Station										n
	1	2	3	4	5	6	7	8	9	10	
<i>Hyla arborea</i> † *	88	77	71	71	59	47	47	71	65	71	10
<i>Rana esculenta</i>	35	29	41	47	41	59	47	71	59	29	10
<i>Bombina bombina</i> † * ‡	41	47	53	41	41	47	12	41	47	47	10
<i>Bufo viridis</i> *	77	53	24	6	18	24	18	71	53	77	10
<i>Bufo bufo</i>	0	0	0	0	29	29	6	6	0	0	4
<i>Rana ridibunda</i>	0	0	0	0	12	12	0	29	12	0	4
<i>Rana lessonae</i>	0	0	6	0	0	0	0	6	0	0	2
Total species / station	4	4	5	4	6	6	5	7	5	4	

Table 2. – Comparison of species observed during visual encounter surveys (V) and road call counts (R). \* Species heard only, not seen during visual encounter surveys.

Species	Ponds							
	A		B		C		D	
	15 April	25 April	15 April	25 April	15 April	25 April	15 April	25 April
<i>Bombina bombina</i>	V	VR	VR	VR	VR	VR	VR	V
<i>Pelobates fuscus</i>	V*	V*		V		V*		V
<i>Bufo bufo</i>		V		V				
<i>Bufo viridis</i>	VR	VR		VR			V	V
<i>Hyla arborea</i>	VR	VR	VR	VR			VR	VR
<i>Rana arvalis</i>				V				
<i>Rana dalmatina</i>	V		V	V	V		V	V
<i>Rana ridibunda</i>							V	V
<i>Rana lessonae</i>		V						
<i>Rana esculenta</i>		VR	V*	VR			V	VR
Total species / survey	5	7	4	8	2	2	6	7

A prime issue to consider is the discrepancies observed between species reported by the two survey methods. The VES confirmed all seven species observed with the RCC method, but also detected three additional species not heard in any of the RCCs along the route: *P. fuscus*, *Rana dalmatina* and *Rana arvalis wolterstorffi*. These species were probably not heard during the RCCs because they call underwater, severely restricting detection distance (ORSZÁGH, 1982, personal observation), and the RCC stations were all more than 50 m from the ponds surveyed by VES. For European anuran species, inter-station distance, call phenology and detection radii should be further investigated in varying habitats (including different assemblages and species natural histories) to determine the likely maximum distance required between RCC stations. A protocol of this nature should also account for frogs with relatively large inter-individual calling distances (e.g., *H. arborea*) to maintain independence of data and avoid double-counting. Furthermore, because human participants generally choose their own routes in volunteer-based RCCs, the sampling design is non-random, resulting in an obvious bias to choose routes where known anuran populations are currently calling, and neglecting inactive sites that potentially could develop future breeding populations. This might produce false estimates of declines by ignoring increasing populations. Conversely, although extensive (random or random-stratified) RCCs may give more accurate indications of breeding population trends, more observers are needed and the latter are more reluctant to conduct randomly selected routes due to the large number of "zeros" likely to be encountered (an admittedly important limitation with random route selection (MOSSMAN et al., 1998; WEIR & MOSSMAN, in press)). As in the North American Amphibian Monitoring Program, striking a balance between hearing the most species during a RCC given the variation in calling distances, and the willingness of volunteers to spend time monitoring anurans is of utmost importance.

In the case of *R. dalmatina* and *R. arvalis wolterstorffi*, the field season may have begun too late, as these are relatively early breeders (PÉCHY & HARASZTHY, 1997) suggesting that the first survey should be conducted in late February or early March. Corresponding with air and water temperatures and life histories of the species present (PÉCHY & HARASZTHY, 1997), three periods are suggested to carry out future RCCs in the study area: early March (*R. dalmatina*, *R. arvalis*), mid-April (*B. bombina*, *B. bufo*, *B. viridis*, *P. fuscus*, *H. arborea*) and mid-May (*R. esculenta*, *R. ridibunda*, *R. lessonae*). More data may be needed to refine this seasonal surveying regime.

An additional limitation with this technique is associated with extraneous noise at RCC stations where birds were calling in large numbers, where frequent traffic noise was experienced, or when wind speed exceeded 20 km/h (BWS > 3). These surveys took longer to conduct and were more frustrating, indicating that volunteers should also be encouraged to choose routes which have minimum extraneous noise from wind, barking dogs, birds, etc. A second factor relating to extraneous noise involves calls of other animal species that sound similar to local anurans. ALSCHIK et al. (1998) demonstrated that both the European nightjar (*Caprimulgus europaeus*) and the horse cricket (*Gryllotalpa gryllotalpa*) emit sounds similar to the territorial call of the green toad (*B. viridis*). The distributions of both of these non-anuran species extends throughout Hungary (BAKONYI et al., 1995), and during the VES conducted at pond D the green toad and the horse cricket were heard calling simultaneously. Therefore, improvements to this protocol should include descriptions of other calling species on instructional materials, and techniques to differentiate these calls. Given the limitations, calling



surveys are unreliable for detecting relatively quiet species or explosive breeders, such as *R. temporaria* and *R. arvalis*, when calling is limited to a short time period (ZIMMERMAN, 1994; PÉCHY & HARASZTHY, 1997; BOWERS et al., 1998).

For many species, however, calls are useful to locate breeding populations, and can be used to detect species presence or estimate the relative abundance of breeding males. On a number of occasions, due to calling underwater or among thick vegetation, *P. fuscus*, *B. bufo* and *R. esculenta* were only detected by sound during VES and not seen, suggesting that in cases where stations are located relatively close to calling individuals, RCCs may be advantageous in detecting species that are cryptic, low in number, or call underwater. This may also hold true for species such as *H. arborea* which have relatively long inter-individual calling distances but migrate during the day from breeding ponds to surrounding vegetation where they can be difficult to see (ORSZÁGH, 1982, personal observation). RCCs can be an effective monitoring tool, especially at sites where visual surveys conducted by walking are logistically difficult, such as: (1) large wetlands; (2) montane lakes with inaccessible shorelines, (3) lakes and wetlands with either soft-bottomed substrates, coarse substrates or extensive woody debris; (4) inaccessible privately owned land. Moreover, when set up as permanent sample sites, RCC routes can yield valuable data not only on local amphibian populations, but also on concurrent changes in habitat components if habitat types are recorded along with data on the species being investigated (COOPERRIDER et al., 1986). These surveys can be conducted by volunteers, and training tapes and manuals make it possible to involve even inexperienced observers (SHIROSE et al., 1997). Conversely, other more comprehensive surveys, including VES, require more expertise, are intrusive in nature, and demand greater levels of time and resources.

Validation of amphibian monitoring programs has been hotly debated at various levels (SHIROSE et al. 1997, DUBOIS, 1998, HFMESATH, 1998). Canadian amphibian monitoring programs have evaluated the accuracy of audio surveys (BERRILL et al., 1992, BISHOP et al., 1997; SHIROSE et al., 1997). Most significantly, these have shown that although calling intensity cannot be considered a true constant-proportion index of abundance, they can be a useful index for populations below a certain size, and to identify trends over extended periods of time. Hence, their potential use in Europe should include analysing species presence/absence at each station, or grouped stations to record trends, with multi-year data sets. HEYER et al (1994) recommended this technique should complement other alternative monitoring methods such as egg or larval counts, or mark-recapture studies, but BENTON (1983) pointed out that such methodologies have their own sets of problems. Nonetheless, parallel trends among several techniques can increase the credibility of conclusions drawn from monitoring efforts. MOSSMAN et al. (1998) accurately indicated that when planning such volunteer-based monitoring programs, competent long term co-ordination must be maintained, dealing with issues including program promotion (e.g., volunteer encouragement), creation of concise and easy-to-understand instructional materials, data compilation and verification, quality control, report generation, and responding to volunteers' enquiries. This crucial component is imperative during the planning phase of any prospective RCC program.

## CONCLUSION

The extent of amphibian distributions in Hungary is poorly documented (GASC et al., 1997). Previous to this study, the KMNP Management Plan recognized only four amphibian species in the Biharugra Landscape Protected Area. However, my RCCs revealed almost two-fold greater anuran species richness including *Hyla arborea* and *Bombina orientalis* both IUCN International Red Data Book species. A national volunteer-based monitoring program employing RCCs, recognizing both their limitations and benefits, would not only be an appropriate complementary approach to monitor taxa indicative of habitats (FARAGÓ & NEMES, 1997), but would also encourage the public at large to conserve and enhance biodiversity to a greater extent across all areas, not just restricted biotopes in protected areas.

## ACKNOWLEDGMENTS

I thank Central European University for financial assistance, Miklós Puky and KMNP personnel including Tamás Zalai and Bela Kalivoda for guidance, Alan Watt and Dan Cogalniceanu for insightful comments on the text, Larisa Gruje for technical assistance, and Lauren E. Brown, Mike Mossman and the other anonymous referees for their helpful comments on the manuscript.

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Corresponding editor: Lauren E. BROWN.

## ***Triturus vulgaris* (Linnaeus, 1758) at its southern limit: distribution on the Peloponnese, Greece, with range extensions from the Central and South Peloponnese**

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This note presents a historical account of the discovery of the Greek smooth newt, *Triturus vulgaris graecus* (Wolterstorff, 1905) in the Peloponnese. Recent observations of populations in the vicinity of Tripoli (Arcadia) and Gythion (Laconia), allow to extend its hitherto known range to the Central and Southeastern parts of the peninsula. This range extension includes the southernmost locality known for that species. The wide Peloponnesian range shown on a map in the *Atlas of Amphibians and Reptiles in Europe* (Gasc et al., 1997) is rejected due to lack of documentation.

### HISTORICAL RECORD

The first published observation of the smooth newt *Triturus vulgaris* (Linnaeus, 1758) in the Peloponnese appeared very early: the *Expédition en Morée* of 1829-1831 mentioned and depicted it under the name of "*Triton abdomnalis* (Latreille, 1800)" (BIBRON & BORY DE SAINT-VINCENT, 1833: 76; pl. 15, fig. 4-5) "Cette espèce se trouve en Morée [= Peloponnese], et notamment aux environs de Modon [= Modhon, Modoni, presently Methoni]" BEDRIAGA (1881: 287) published this mention, identifying the species as "*Triton palustris* (Linnaeus, 1758)" The second observation, that of a female by Dr. Marán in April 1936 in Kalavryta, was published by ŠTĚPÁNEK (1944: 123), who identified the newt as *Triturus vulgaris graecus* (Wolterstorff, 1905) (type locality: Corfu - Kerkyra). In recent annotated lists of amphibians of the Peloponnese (BRINGSØE, 1986; KHYMAR, 1986a), or in that for the genus *Triturus* in Greece (SOTIROPOULOS et al., 1995), the range of *Triturus vulgaris* on that peninsula appeared to be scattered and limited to the northerly regions with one exception, that of Methoni.

BRINGSØE (1986: 282, 311) gave the following localities, with the references: Kalavryta [= Kalavryta] (ŠTĚPÁNEK, 1944), "approx. 8 km N of Didyma", from eggs, but with reservation

for their identity (ADEMA & IN DEN BOSCH, 1980) and "Kalogria Wood" (personal communication to the author by Chondropoulos from 1985). Besides, Bringsøe called attention to the old record at Methoni. Later, in 1994, the same author (H. B.) visited, together with Jørgensen, the forest of Kalogria (more accurately Strofilia) where they observed many larvae (BRINGSØE, unpublished, JØRGENSEN, 1995). KEYMAR (1986a: 5) mentioned only Kalavryta, Methoni and Didyma, without further details, and the distribution map published by NÖLLERT & NÖLLERT (1992: 203) included these same three localities, showing simultaneously a question mark for the remaining part of the Peloponnese. In our opinion, some doubt could also apply to Methoni, unconfirmed locality for 170 years. However, on the present basis we are unable to judge whether *T. vulgaris* still occurs around Methoni as we are not aware to what extent the area has been investigated, so far we have not seen any reports describing sufficiently detailed surveys which have been carried out at the right time of the year (as to the proper season; see also further down this text).

In a second article, KEYMAR (1986b: 14, 19, 35) stated: "Die subspezies *graeus* kommt auf den vier großen Ionischen Inseln", i.e. Kerkira [= Corfu], Lefkas, Kephalonia [= Kefalonia] and Zakynthos, whereas on the joined map this last island was indicated only with a question mark. On the other hand, the same map seems to show a new point in the northwestern part of the peninsula, to the north of Pyrgos, in the nomos (municipality) of Ilios [= Ileia]. We think that actually Keymar wanted only to indicate the presence of this newt on the nearby Peloponnese in a quite superficial way. CLARK (1989: 9), in a check-list of the herpetofauna of the Argo-Saronic Gulf region, repeated the Didyma record as published by Bringsøe, without adding any new data. Finally, one of us (BRINGSØE, 1994: 354-358) presented a new locality for *Triturus vulgaris graeus*, "5-6 km east of Kertezi, east of Mt. Erymanthos . . . altitude 745 m" [that is, approx. 10 km southwest of Kalavryta], where this *Triturus* taxon is sympatric with *Triturus alpestris* (Laurenti, 1768)<sup>1</sup>.

SOTIROPOULOS et al. (1995) compiled an exhaustive study of the geographic distribution of the genus *Triturus* Rafinesque, 1815 in Greece, based on a large bibliography (primary or secondary sources), quoting even... Aristotle! Thus, these authors listed the localities of Didyma (BRINGSØE, 1986), Ileia [Ilios] without any details (KEYMAR, 1986b; we gave our interpretation of this "locality" above), Kalavryta (ŠTĪPANIK, 1944), Kalogria (BRINGSØE, 1986), Kertezi (BRINGSØE, 1994) and Modon (BIBRON & BORY DE SAINT-VINCENT, 1832). Further, they added Patras, with reference to BURISCH & ZONKOV (1941: 223). This last reference originates back from WERNER (1898), however, this author had in fact written (see WERNER, 1899: 16): "Kryoneri, Arkananien, gegenüber Patras". In doing so he clarified the location of Kryoneri (a common toponym in Greece). Thus, only Kryoneri (nomos of Arkanania) is a valid locality in this connection, and Patras has obviously been included by mistake. The localities of Kalavryta, Kalogria and Kertezi are situated in the north-northwest of the Peloponnese, in the nomos of Achaia [= Akhaia] Didyma (northeast, in Argolis) has a degree of uncertainty as the record is entirely based on eggs which were impossible to identify properly. Likewise Methoni (southwest, in Messenia) has not been confirmed since its original mention (see above).

1 About *Triturus alpestris* in Greece: see BRIEF & PARINE (1988a,b) and BRINGSØE (1994)

As for the *Atlas of Amphibians and Reptiles in Europe* (GASC et al., 1997: 88), on one hand it depicts the Peloponnese almost completely covered with dots of recent presence (observations after 1970; one dot corresponds to a grid of  $50 \times 50$  km), and absence being only conspicuous for the eastern "finger" (the Monemvasia area), on the other hand, the islands of Kefallinia and Zakynthos are also covered, unlike Lefkas (fig. 1). However, the sources for such a comforting distribution have not been published. Patrick Haffner, who participated in the elaboration of this *Atlas*, and especially in the collecting of data, has clarified to us

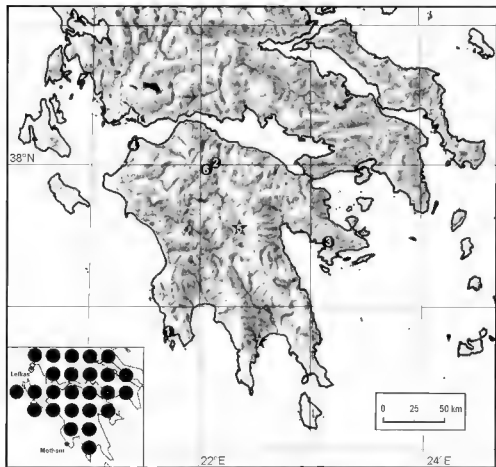


Fig. 1 Southern Greece including the Peloponnese. Localities in the peninsula mentioned in the text where the presence of *Triturus vulgaris* is or was established, in chronological order: (1) Methoni (1829-1831), (2) Kalavryta (1936), (3) Didyma (1980), (4) Kalogria (1985), (5) Kapasia (1987), (6) Kertez (1994), (7) Gyntion (2001). The stars mark the new localities, dots indicate other localities which have previously been published. The map in the inset shows the range of *Triturus vulgaris* (large dots), according to GASC et al. (1997: 88). Two previously published localities (small diamonds) were omitted in this map: the island of Lefkas (Lefkada), although the presence of the newt was mentioned since 1938 (see SOULIOTIS et al., 1995: 76), and the old locality of Methoni (Modnon). We reject this distribution map (see text).

(personal communication, 15.X.2001) that the set of these "filled grids" was supplied by Sofianidou, but that the original data are retained with her<sup>2</sup>. It is highly regrettable that no document (publications, pictures or voucher specimens for example) authenticates such findings, and we are unaware of the precise basis for selecting these Peloponnesian "localities" for *T. vulgaris*, which, in our opinion, makes the *Atlas* unreliable (on this subject read the detailed criticism by DUBOIS, 1998). Therefore, nothing allows us to accept this new distribution of the smooth newt on the Peloponnese, and we reject it, awaiting possible clarification and documentation. Heinz Grillitsch (personal communication, 16 X 2001) pointed out that the same questions can be also asked, and for the same reasons, about the distribution of *Pelobates syriacus* Boettger, 1889 on this peninsula.

### RECENT OBSERVATIONS

Since 1978, one of us (H B) has made regular field excursions on the Peloponnese, with the aim to clarify eco-ethology and chorology in terms of herpetology. On April 23, 1987, he discovered a population of *Triturus vulgaris graecus* (two males and three females observed; fig. 2b) in the vicinity of Tripoli [= Tripolis, Arcadia], more exactly 4 km southeast of Kapsia [- Kapses, Kapsas, Kapsias; i.e. approx. 10 km north of Tripoli; altitude approx. 680 m], but this discovery has so far not been published. The environment consisted of some big ponds, situated in pastures, olive groves and other kinds of arable fields (fig. 2a). Among syntopic species, he noticed *Rana* cf. *ridibunda* Pallas, 1771<sup>3</sup>, *Hyla arborea* (Linnaeus, 1758), *Pelobates syriacus balcanicus* Karaman, 1928 (a large tadpole) and *Emys orbicularis hellemica* (Valenciennes, 1832) In the very nearby terrestrial habitat, *Testudo hermanni boettgeri* Mojsisovics, 1889, *Podarcis taurica ionica* (Lehrs, 1902) and *Mulpolon monspessulanus insignitus* (Geoffroy, 1829) were recorded as well. The same observer returned to this locality on April 25-26, 1994, but his attempts to find *T. vulgaris* by sweeping a net in the water were in vain. However, a third visit (April 18, 2001) proved successful: after considerable searching one male and two females were found. It is possible that the low number of specimens found in the water was due to the relatively late time of spring regarding each of the three years. This is the first mention of the presence of the smooth newt on the Central Peloponnese. The new locality is situated roughly halfway between Kalavryta and Didyma

During fifteen trips of one to three weeks duration to the southern Peloponnese, spread over ten years, and dedicated primarily to the study of populations of *Testudo weissingeri* Bour, 1995, the two other authors (R. B & M. V) carried out herpetological investigations in a variety of habitats in the northern part of the area called Mani, especially inside a triangle joining Kalamata, Areopoli and Gythion [= Yithio]. During these investigations, more than

2 Sofianidou published in the same period a "check-list" of the tetrapods of Greece (SOFIANIDOU, 1996), where this species is only briefly mentioned in the text, maps have only been arranged for two other Greek species of the genus *Triturus*, *T. ulpestris* (Laurenti, 1768) and *T. karchmi* (Strauch, 1870), whereas *T. carnifex* (Laurenti, 1768) has been entirely omitted from the publication. We can add that in this work, the Peloponnesian distribution map of *Lacerta graeca* Bedriaga, 1886 is also too wide and that of *Chalcides ocellatus* (Forsskål, 1775) is by far too extensive.

3 The taxon is probably *Rana kurtmuelleri* Gayda, 1940, older subjective synonym of *Rana balcanica* Schneider & Smissch, 1992 (see DUBOIS & OHLER, 1995, 175)



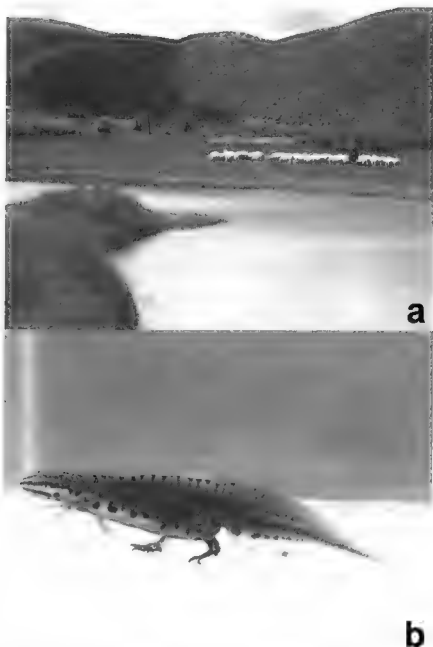


Fig. 2a Habitat of *Triturus vulgaris gracilis* 4 km southeast of Kapsia (north of Tripoli), altitude about 680 m. Taken on 18 April 2001 (photo Henrik Bringsøe)

Fig. 2b Male *Triturus vulgaris gracilis* recorded 4 km southeast of Kapsia (north of Tripoli), 23 April 1987. Notice the tail filament and the unspotted lower margin of the tail fin (photo Henrik Bringsøe)



Fig 3a View of the habitat where the young newts were discovered, 5 km west-southwest of Gythion, the southernmost known locality for *T. vulgaris*. The bed of the Zmnos (invisible) stretches on the left, the pond on the right, both are dried out. Taken on 1<sup>st</sup> October 2001 (photo Roger Bour)

Fig 3b-c Young *Titurus vulgaris graecus* found 5 km west-southwest of Gythion 1<sup>st</sup> October 2001 (photo Roger Bour).

950 individuals of *Testudo weissingeri* were measured and marked, and isolated micropopulations of "dwarf" *Testudo hermanni* Gmelin, 1789 were discovered. Additionally, the sand boa *Eryx jaculus* (Linnaeus, 1758) proved to be much less rare than was previously thought (see BRINGSØE, 1986), due to the observation, under favourable atmospheric conditions (warm and wet weather), of more than fifty specimens (young and adults) within about 20 hectares of light but stony soil, on two olive groves. Encountering tailed amphibians was on the other hand exceptional. The fire salamander *Salmandra salamandra* (Linnaeus, 1758) was observed in or close to mountain streams (torrents) of the Mt Taygetos range. The southernmost localities are the monastery of Agios Samouil (36°53'N, 22°17'E; altitude approx. 750 m and above; larvae and one adult), and the eastern slope of the Mavrovounia, in Agios Panteleimonas (36°54'N, 22°21'E; altitude approx. 1500 m; larvae).

However, during the latest field trip (September 20-October 10, 2001), a second urodele species was found (on October 1), more precisely, four young of *Triturus vulgaris* (Linnaeus, 1758) in their terrestrial phase (total length approx. 35 mm; fig. 3b-c), most probably belonging to the subspecies *graecus* (Wolterstorff, 1905). These newts were gathered, hidden under a slab of about 30 × 50 cm in size, on the sloppy and wooded bank of a dried pond. No more newts were found under other objects like rocks, tree stumps and various garbage. The pond, about 20 × 40 m, extends along the small river named Zminos, flowing like an oued, which runs down from the south of the Taygetos, and which feeds it with water by infiltration. In spring, the water level reaches 100 to 150 cm. This place is partly covered with hydrophilous vegetation and trees, but it is used mainly as a rubbish dump, with many piles of garbage half submerged during the wet season (fig. 3a). Crops extend into the surroundings. The herpetofauna of this semi-aquatic habitat has so far been observed to also consist of *Rana* cf. *rubivanda* Pallas, 1771 (see note above), *Hyla arborea* (Linnaeus, 1758), *Emys orbicularis hellenica* (Valenciennes, 1832), *Mauremys rivulata* (Valenciennes, 1833) and *Natrix natrix persa* (Pallas, 1814). In the surrounding terrestrial habitat, *Eryx jaculus turicus* (Olivier, 1801) and *Testudo hermanni* subsp. were also registered. The exact place of these observations is situated between Nea Marathea and Agios Vasilios (36°45'N, 22°31'E; altitude approx. 10 m), roughly 5 km west-southwest of Gythion (Lacoma). Unfortunately we have to add that this place, as many others, is used as a dump for garbage, and that pumps feeding irrigation networks are more and more wide-spread along the banks of the Zminos: the future of this habitat and its fauna is therefore very questionable<sup>4</sup>.

## DISCUSSION AND CONCLUSION

With these new records, in total seven localities inhabited by *T. vulgaris* are known from the Peloponnese (fig. 1): (1) Methoni (Messenia), (2) Kalavryu (Achaea), (3) Didyma (Argolis), (4) Kalogria (Achaea), (5) Kapsta (Arcadia), (6) Kertzei (Achaea), and (7) Gythion (Laconia). Five of them were discovered during the last twenty years. Results are encourag-

4 More generally, the problem of water supply is going to arise more and more in the Peloponnese, mostly in connection with the increasing development of these irrigation networks and catchments made into rivers, mostly temporary, or straight into springs.

ing, because it is a priori surprising to notice southern range extensions of an animal species which depends on aquatic environments, in a region where aridity seems to progress at an alarming rate, simply due to major destructions of the environment throughout Greece.

Several explanations, in part complementary, may be put forward to explain these discoveries. It is likely that researchers as well as the searches are today more numerous and more accurate. It should be kept in mind that *T. vulgaris* leaves its breeding pond relatively quickly, after egg-laying, to adopt a fully terrestrial life. The metamorphosed young return to the water only after several years, in spite of the drying of ponds, puddles or ditches during summer, this species still manages to survive such hostile environments. We may also postulate that on the Peloponnese – i.e. the very south of its range – *T. vulgaris* most probably breeds earlier in the year and thus leaves the water earlier than in the north, but that will need to be properly documented. The phenology of *T. vulgaris* in another southern range, the Izmir region of West Turkey, has already been established: adults are aquatic from around December through April (SCHMIDTLER & SCHMIDTLER, 1967, contrary to ÖZETI, 1964, who extends the aquatic phase up to June). We think it probable that the South Greek populations of *T. vulgaris* exhibit a similar tempo of activity. That may explain why it was so difficult to record the species in the water of the Kapsia locality at the end of April. More surprising is the fact that *T. vulgaris* and *T. alpestris* were found to be abundant in the ditches at the Kertezi locality on 17-18 May (BRINGSØE, 1994). For comparison, in Central Europe adult *T. vulgaris* usually leave the water in about late June to early July (NÖLLER & NÖLLER, 1992). Another possible explanation for this apparent sporadic range involves a positive human action, i.e. a wilful introduction, or as stowaway. For instance, the last found locality is not far from a sports ground and is used as a garbage: this place is frequently visited. Finally, nocturnal activity of the newts on the ground does not favour observation of individuals in their terrestrial phases. Early April and earlier may be best to register *T. vulgaris*.

Although *T. vulgaris* may be more common on the Peloponnese than what is usually believed, we expect that it exists in rather sparse and isolated populations. Generally, the Peloponnese is very dry and seems to have relatively few and scattered suitable freshwater habitats. For *T. vulgaris* the landscapes of the Peloponnese will appear fragmented and it may be difficult to colonise and re-colonise new habitats. Other amphibians like *Hyla arborea*, *Bufo bufo* (Linnaeus, 1758), *B. viridis* Laurenti, 1768 and *Rana ridibunda* s. l. are known to be able to migrate over longer distances and are more mobile on land than *T. vulgaris* (see for instance the individual species accounts in GÜNTHER, 1996). These four anuran species are common and widely distributed on the Peloponnese and often breed in man-made habitats of standing freshwater (BRINGSØE, 1986).

## RÉSUMÉ

Les découvertes successives du Triton ponctué grec, *Triturus vulgaris graecus* (Wolterstorff, 1905), dans le Péloponnèse, sont rappelées dans leur chronologie. Cette note précise la repartition actuellement connue du Triton ponctué pour l'ensemble de la presqu'île et révèle la présence de cette espèce dans le centre (environs de Tripoli, Arcadie) et le sud-est (environs de Gythion, Laconie, localité la plus méridionale connue). La distribution

présentée dans l'*Atlas of Amphibians and Reptiles in Europe* (GASC et al., 1997), couvrant la majeure partie de la péninsule, est rejetée car elle n'est pas étayée par des spécimens ou des données précises

### ACKNOWLEDGMENTS

We warmly thank all the following people or institutions. In Greece, Kostas et Gioula Georgouleas, from Kardamili, for their welcome and their help, especially during hard times, and George I Handrinos, from Athens, for his confidence and his support. In Austria, Heinz Grillitsch, from Vienna, and in France, Patrick Haffner, from Paris, for their valuable information and suggestions in connection with the *Atlas*. The field work was undertaken with permits (2001: permit # 103866/4506) delivered by the Ministry of Agriculture (General Secretariat of Forests and Natural Environment) in Athens. The manuscript was improved thanks to the thorough revisions by Michel Breuil and Alain Dubois (Muséum national d'Histoire naturelle, Paris), by Jarmo Perälä (University of Bristol) and to the comments of the referees.

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Corresponding editor: Alain DUBOIS

## Origin and development of the vent tube in two species of the genus *Bufo*

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The vent tube of *Bufo arenarum* (medial configuration) and *Bufo fernandezae* (dextral configuration) tadpoles is derived from the ectodermal portion of the embryonic intestine exit. Ciliated epithelial cells provided by the epidermal surface (skin) stay as proctodeal mark inside of the cloaca during the larval development. The distal region of the embryo's proctodeum and larval epidermis contribute to form the definitive vent tube. The presumptive intestinal exit is present at Gosner stage 19, the anatomical cloaca is fully formed at stage 20 or early 21 (*urodaeum* and *proctodaeum*). After the common features are completed in *B. fernandezae*, the final configuration of the dextral vent tube becomes evident at stage 23 by the formation of a fold on the right side of the ventral fin. At stage 25, each type of vent tube takes its definitive shape and becomes functional. In both cases, a similar tissue organization of the cloaca and vent tube was found. For comparative purposes, the vent tubes of different types of larvae (1, 2 and 3 sensu ORTON, 1953) in stages 25-26, and with medial configuration, were examined at least, by one of the techniques indicated. They showed similar structural organization, among them and with the bufonids examined in this study; however, two layers of epithelial tissue separated by mesenchyme were always present.

### INTRODUCTION

Tadpoles' vent tubes may have various configurations and may or not be associated with the anterior portion of the ventral fin. This tube usually projects from the medial part of the ventral body wall on the sagittal plane (AFTIG & JOHNSTON, 1989; McDIARMID & AFTIG, 1999). Modifications of the distal parts of the ventral and/or right wall eventually produce a tube that opens either parallel with (medial) or to the right (dextral) of the plane of the ventral fin (AFTIG & McDIARMID, 1999). The presence of such an extension for focal transport is unique to anuran tadpoles. This larval feature develops during late embryonic stages, it has no adult derivatives, and it atrophies at about the same time when the front legs emerge through the *operculum* (TAYLOR & KOLLROS, 1946; DEL CONTE & SIRLIN, 1951; GALLÉN & HOULFON, 1951; VAN DIJK, 1959; ECHEVERRÍA, 1998).

HUETTNER (1948) described the cloaca of *Rana* as continuous with the *rectum*, and as formed partly by endodermic and ectodermic (*proctodaeum*, sensu GADOW, 1887, fide VAN DUK, 1959) tissues. Among bufonids, the formation of the proctodeal pit has been reported by several authors (MARCHETTI, 1919; RONDININI, 1928; KAGAWA, 1932; SCHECHTMAN, 1939, for *B. bufo*, fide VAN DUK, 1959) without comments about its relationship with vent tube development.

In an attempt to supply the comparative morphological information required to evaluate the assumption that the presence of two vent tube configurations for the same function does not necessarily mean different anatomical and/or histological features, I report on the ontogeny of the vent tube and the tissues that give rise to the vent tube.

### MATERIAL AND METHODS

A total of 100 embryos and tadpoles of *Bufo arenarum* and 20 of *Bufo fernandezae* were used to study the internal and external development of the vent tube between GOSNER (1960) stages 17 and 25. Embryos were preserved in buffered 10% formalin; *B. arenarum* embryos and larvae were fixed every 60 min. The specimens were examined with incident lighting and with a scanning electron microscope (SEM), after critical-point drying and gold-palladium coating. *B. fernandezae* specimens were reared in the laboratory for taking photographs in vivo of the main changes of the vent tube development.

For light microscopic examination, tadpoles and embryos were dehydrated intact in an alcohol series, embedded in paraffin (56-58°C), sectioned in transverse, sagittal and frontal sections of 4 or 6  $\mu$ m, and stained with hematoxylin-eosin or Masson's trichrome (MARTOJA & MARTOJA-PIERSON, 1970). Histological terminology follows WELSH & STORCH (1976). Pertinent drawings of the development were drawn with a camera lucida. For SEM observation, three tadpoles of *B. arenarum* (stages 27, 31 and 32) and four specimens of *B. fernandezae* (stages 23, 24, 25 and 26) were dissected to study the inner walls of the cloaca and vent tube.

The description of *B. fernandezae* vent tube development will be done in short form to explain differences from *B. arenarum*. For comparative purposes, I examined the vent tubes of tadpoles of different types (ORTON, 1953) of larvae, in Gosner stages 25-26, of *Xenopus laevis* (Orton type 1), *Gastrophryne carolinensis* (Orton type 2), *Axcaphus trueri* (Orton type 3), at least by one of the techniques described.

The term inner cloaca is used to indicate that region of the cloaca which is situated in the pleuroperitoneal cavity.



## RESULTS

THE MEDIAL VENT TUBE OF *BUFO ARENARUM*

The first external evidence of the formation of the exit part of the gut appears at stage 16 as an indistinct pit on the posteroventral margin of the body. This pit is the incipient proctodeum. Ciliated epidermal cells occur in the wall of this depression (fig. 1a). Sagittal sections show an ectodermic invagination on the posterior part of the embryo. There is no lumen in the hind-gut region (fig. 1b).

At stage 18, there is a definite depression although the proctodeum remains closed. All cells have yolk platelets, and cellular limits are not clear because of these platelets. In addition these epithelial cells have black pigment in the peripheral cytoplasm of their distal edges. Proctodeal cells appear more darkly pigmented, and these cells have long cilia that project into the lumen (fig. 1c). The cloaca *sensu stricto* is not yet formed. Opisthonephric kidneys buds and pronephric ducts are not evident posteriorly, although the pronephric ducts have begun differentiation anteriorly.

By stage 19, this proctodeal pit is located slightly anterior to the beginning of the ventral fin anlage (fig. 1d). During stage 19, the cloacal membrane persists, that separates the intestine *per se* from the outside. Below the epidermis there are a few dispersed mesodermal cells. The proctodeum is separated from the endoderm by a layer of tissues hardly defined that constitute the "cloacal membrane" (ectoderm, endoderm, and poorly evident to absent mesenchyme) (fig. 1e). The proctodeum cavity is funnel-like, without connection to the inside of the gut, it is held by the dorsal part of the ventral fin anlage (fig. 1f). Cells still have many yolk platelets that makes the delineation of individual cells difficult. Black pigment remains in these cells.

At stage 20, the pronephric ducts finally connect to the distal part of the hind gut, and the functional larval cloaca is formed. A constriction in the cloaca marks the point of union between ectoderm and endoderm, and presence/absence of ciliated cells of the epidermis mark the limit between both tissue sources (fig. 2a-b). The proctodeal epithelium is stratified, composed of two layers of cuboidal to polyhedral cells that rest on a basal layer.

At stage 21, an incipient peritoneum is forming in the pleuropertitoneal cavity. Cellular surface specializations of the intestine are absent. A ventral constriction develops, that marks the posterior part of the ventral zone of the body (fig. 2c). As the constriction grows inward, the vent cylindrical mass is more evident on the ventral side and separates from the body. The inner part of the larval cloaca has been composed by the *urodaeum* and part of the *proctodaeum*, before the ventral fin began to grow, and the tadpole's intestine became functional.

By stage 22, the body and tail are upwards slightly, and the vent opening occurs where the ventral fin contacts the body. Mesenchymal cells grow between the tail muscles and the dorsal wall of the proctodeum. The ventral fin grows at the expenses of the mesenchyme placed below the tail muscles, and in continuity with the posterior margin of the external orifice of the intestine (fig. 2d).

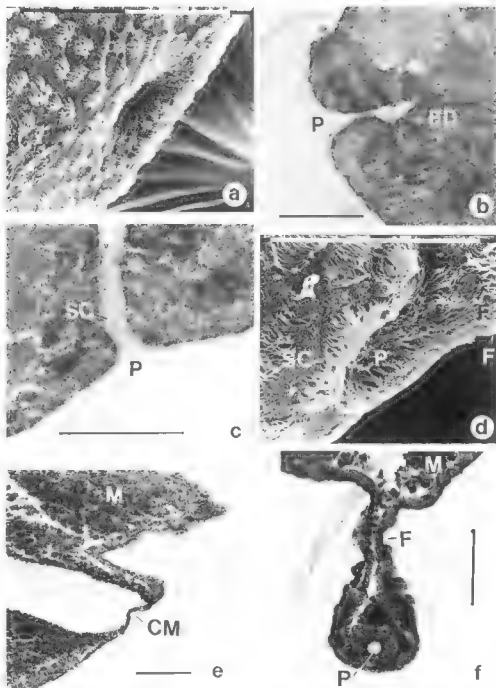


Fig. 1. (a) Scanning electron photomicrograph of the proctodeum pit, from a Gosner stage 17 embryo of *Bufo arenarum*. Left lateral side view. Scale line: 100  $\mu$ m. (b) Sagittal section through the proctodeum invagination of a stage 16 embryo of *B. arenarum*. ED, endoderm; P, proctodeum. Stained with haematoxylin-eosin. Scale line: 100  $\mu$ m. (c) Sagittal section through a stage 18 embryo of *B. arenarum*. Cilia (SC) in the lumen of the proctodeum (P). Stained with haematoxylin-eosin. Scale line: 100  $\mu$ m. (d) Scanning electron photomicrograph of the proctodeum pit (P), from a stage 19 embryo of *B. arenarum*. Left lateral side view; F, ventral fin anlage; SC, cilia. Scale line: 100  $\mu$ m. (e) Sagittal section of the cloaca membrane in a stage 19 embryo of *B. arenarum*. CM, cloaca membrane; M, tail muscles. Stained with haematoxylin-eosin. Scale line: 100  $\mu$ m. (f) Cross section of the proctodeal region in a stage 19 embryo of *B. arenarum*. Cilia in the lumen of the proctodeum (P); F, ventral fin; M, tail muscles. Stained with haematoxylin-eosin. Scale line: 100  $\mu$ m.

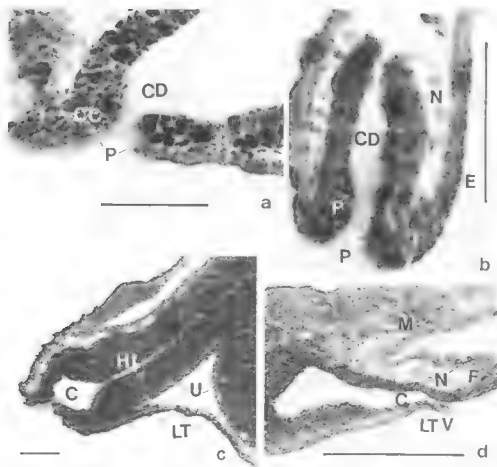


Fig. 2. (a) Sagittal section of cloacal region in a stage 20 embryo of *B. arenarium*. CD, endodermal cavity of the embryonic cloaca, CC, ciliated cells; P, proctodeum. Stained with Masson's trichrome. Scale line: 100  $\mu$ m. (b) Cross section of the proctodeal region of a stage 20 embryo of *B. arenarium*. CD, endodermal cavity of the embryonic cloaca; E, epidermis; N, mesenchyme; P, proctodeum. Stained with Masson's trichrome. Scale line: 100  $\mu$ m. (c) Sagittal section of the posterior intestine and cloaca of a stage 21 embryo of *B. arenarium*. C, cloaca; HI, posterior intestine; LT, ventral constriction; U, peritoneum trace. Stained with Masson's trichrome. Scale line: 100  $\mu$ m. (d) Sagittal section of the inner cloaca (C) and vent tube anlage (V) of a stage 22 embryo of *B. arenarium*. F, ventral fin; LT, ventral constriction; M, tail muscles; N, mesenchyme. Stained with haematoxylin-eosin. Scale line: 100  $\mu$ m.

At stage 23, the only external evidence of the vent tube anlage is a cylindrical mass of the epidermis that includes the presumptive hind limb buds (fig. 3a). Viewed externally, this mass is continuous with the skin of the body, and limb buds begin to separate from the vent structure at early stage 23. The aperture of the vent tube is slightly opened. At the end of this stage the limit between the body and the vent tube anlage is evident (fig. 3a). Ciliated cells are

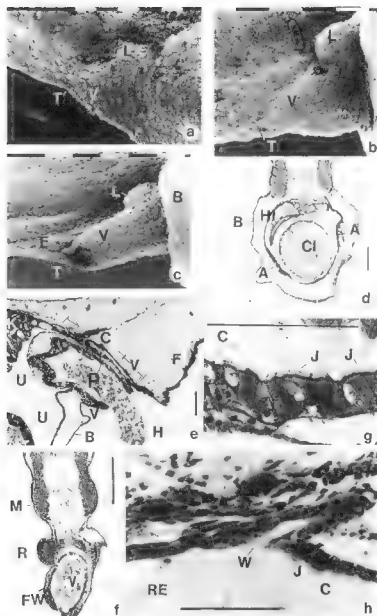


Fig. 3. (a) Scanning electron photomicrograph of the vent tube (V) and leg (L) buds in early stage 23 of *B. arenarum*. T, vent tube exit. Scale line: 100  $\mu\text{m}$ . (b) Scanning electron photomicrograph of the vent tube (V) and the leg bud (L) in lateral view from stage 24 of *B. arenarum*. T, vent tube exit. Scale line: 100  $\mu\text{m}$ . (c) Scanning electron photomicrograph of the vent tube (V) from a stage 25 of *B. arenarum*. B, body, F, ventral fin, L, leg bud, T, vent tube exit. Scale line: 100  $\mu\text{m}$ . (d) Cross section of a stage 25 tadpole of *B. arenarum*. A, rectus abdominis muscle; B, body wall, CI, inner cloaca, HI, posterior intestine. Stained with Masson's trichrome. Scale line: 100  $\mu\text{m}$ . (e) Sagittal section of distal part of the cloaca (C) and vent tube (V) of a stage 26 tadpole of *B. arenarum*. B, body wall, F, ventral fin, H, fecal string, K, kidney, P, proctodeum zone, U, peritoneum. Stained with Masson's trichrome. Scale line: 100  $\mu\text{m}$ . (f) Cross section of a stage 26 tadpole of *B. arenarum*. FW, fin wall, M, tail muscles, R, right posterior limb, VT, vent tube. Stained with Masson's trichrome. Scale line: 300  $\mu\text{m}$ . (g) Sagittal section of the ciliated epithelium in the proctodeum zone of the cloaca (C) of a stage 26 *B. arenarum*. J, ciliated surface. Stained with Masson's trichrome. Scale line: 50  $\mu\text{m}$ . (h) Sagittal section of the dorsal wall of the distal part of the rectum (RE) next to the cloaca (C) of a stage 26 *B. arenarum*. J, ciliated surface, W, nephric duct orifice. Stained with Masson's trichrome. Scale line: 50  $\mu\text{m}$ .

still present on the skin, the tail increases in length, and the ventral fin is well formed and attached to the posterior margin of the exit orifice (fig. 3a). The vent tube anlage grows in distal direction, forming its external tube, in expenses of the epithelial and mesenchymal cells around it.

At stage 24, the vent tube and limb buds are evident. The tube is conical, and the terminal aperture, which may be oval or circular, is open. The vent tube is still blocked inside (fig. 3b). At stage 25, the vent tube is fully developed and becomes functional. The aperture is circular and faces ventrally. The anterior edge of the ventral fin is connected to the posterior side of the vent tube (fig. 3c). Transversal section of the posterior region of the tadpole's body shows that the cloaca arises between the *rectus abdominis* muscles (fig. 3d) They are attached to the posterior wall of the tadpole's body, and they are the only muscles next to the inner cloaca.

After its final organization in stage 25, the vent tube grows continuously. It grows during the later stages in expenses of the epidermis and dermis (fig. 3e) Epithelial and mesenchymal tissues of varying thickness are surrounding the vent tube cavity and form the ventral fin too (fig. 3f) The epidermis has definitively lost the cilia at stages after 25, but the surface of the proctodeum epithelia region has not (fig. 3g).

Several features in *B. arenarum* are important to be commented. The microvilli of the intestine appear at stage 24, in the anterior gut and midgut. The epithelium of the *rectum* is formed by a single layer of prismatic to cuboid cells with a low brushborder. Ciliated cells are only found behind the place where the nephric ducts enter the cloaca (fig. 3h). The *proctodaeum* tissues do not make structural changes, at least during stages that have been covered in this study (17 to 32).

#### THE DEXTRAL VENT TUBE OF *BUFO FERNANDEZAE*

At stages 19 to 21, the embryo develops the cloacal exit presumptive zone and the dorsal and ventral fin buds (fig. 4a). At stage 22, in the posterior and ventral zone of the body, the vent tube anlage is evident (fig. 4b). At stage 23, on the right side of the ventral fin next to the body appears a fold, which holds the incipient vent tube (fig. 4c). At stages 24-25, the median and distal part of the vent tube is formed. The tube takes its definitive shape and is functional at stage 25. The configuration is dextral and marginal to the ventral fin (fig. 4d).

Histological preparations of the vent tubes of *B. arenarum* and *B. fernandezae* showed that the last part of the alimentary tract (*rectum* and proximal part of the cloaca) runs on the left side of the pleuroperitoneal cavity, arising with medial configuration between the *rectus abdominis* muscles (fig. 3d, 5a). In both medial and dextral tubes, the proximal part of the vent tube is placed between both walls of the ventral fin (fig. 3f, 5b-c); medial and distal parts of the vent tube of *B. fernandezae* are placed on the right wall of the fin, and are attached to its ventral edge (fig. 5d-f).

The definitive vent tube in *B. arenarum* and *B. fernandezae* is composed of an inner layer of cuboidal epithelial tissue, surrounded by an outer wall (epidermis on most lateral and ventral sides) separated by mesenchyme of different thickness (fig. 3e f, 5) This inner epithelium contains secretory cells, and is partially covered by ciliated cells on its proximal part, next to the inner cloaca.

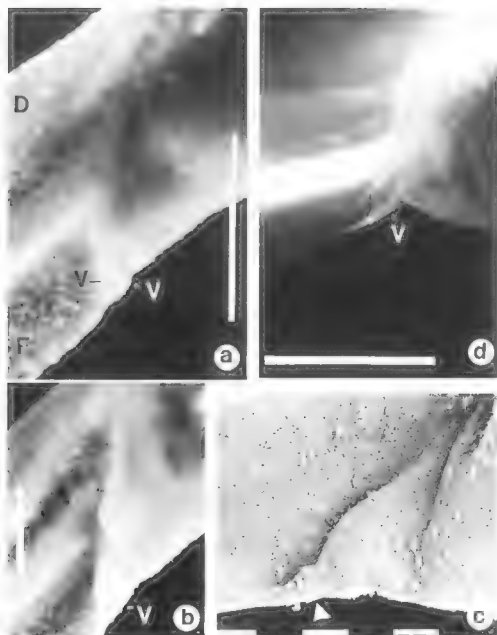


Fig. 4 External morphology of the vent tube (V) of *Bufo fernandesi*. Photographs taken in vivo. - (a) Stage 21. Scale line = 1mm. - (b) Stage 23. Scale line = 1mm. - (c) Scanning electron microscope photomicrograph of the vent tube anlage (arrow), Stage 24. Scale line: 100  $\mu$ m. - (d) Stage 24. Scale line = 1mm. D, dorsal fin; F, ventral fin, V, vent tube

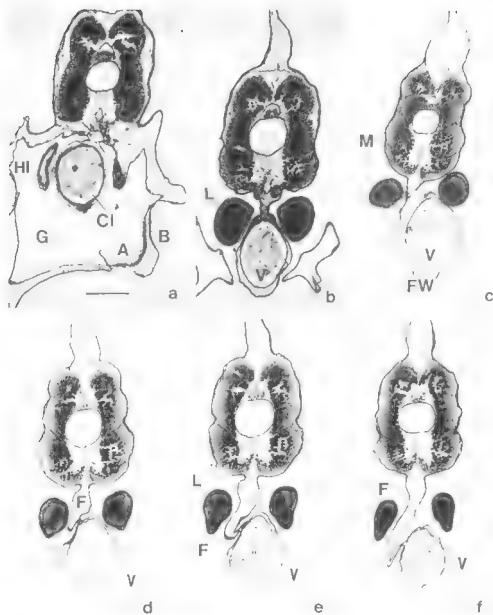


Fig. 5. Cross section of the posterior part of the body and tail of a tadpole of *B. fernandezii* Stage 34. (a) Cloaca in the interior of the pleuroperitoneal cavity (G). (b-d) Median region of the vent tube (e-f) Distal region of the vent tube. Scale line, 300  $\mu$ m. A, *rectus abdominus* muscle; B, body wall, Cl, inner cloaca, F, ventral fin, FW, fin wall, HI, posterior intestine; L, posterior leg buds, M, tail muscles, V, vent tube. Stained with Masson's trichrome. Scale line: 300  $\mu$ m.

Cloacas of *B. arenarum* and *B. fernandezae* tadpoles dissected after the critical point procedure and observed by SEM confirmed that the ciliated cells are present in the distal portion of the inner cloaca and in the proximal part of the vent tube. Ciliated cells are absent on the distal wall of the vent tube after stage 26 (fig. 6a-c). In stage 25, microvilli of the surface of the intestines next to the cloaca are low and continuous (fig. 6c). In intestines full with food, the brushborder is more evident and dense than in the *rectum* and in the proximal part of the inner cloaca

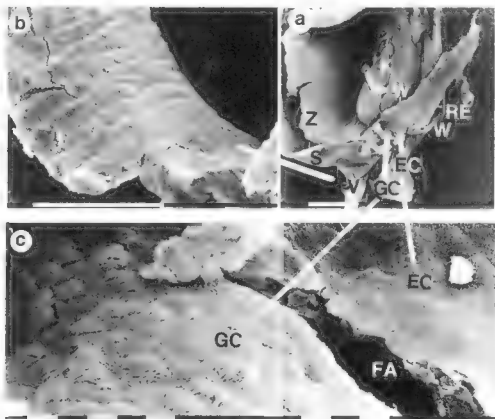


Fig. 6. - (a) Scanning electron microscope microphotograph of a dissected stage 32 tadpole of *B. arenarum*: EC, region of plane epithelium in the inner cloaca, GC, region with ciliated epithelium of the proctodeum, K, kidney, RE, hind intestine or rectum, S, skin, V, vent tube, W, nephric duct orifice, Z, parietal peritoneum. White scale line 1 mm. (b) Detail of the distal margin of the vent tube with smooth surface in a stage 32 tadpole of *B. arenarum*. White scale line 0,1 mm. (c) Detail of the surface of the cloacal epithelium. EC, region of plane epithelium in the inner cloaca, FA, artifact, GC, region with ciliated epithelium of the proctodeum. White scale line 10  $\mu$ m.



The larval cloaca of these tadpoles, could be "divided" in two anatomical parts, *urodaeum* and *proctodaeum*. Urinary bladders are not formed earlier than at stage 32. The region of the larval cloaca into which the nephric ducts open (*urodaeum*) is endodermal. The following region of the cloaca is ectodermal (*proctodaeum*) (fig. 3h).

Concerning the vent tube organization in the larvae of *Xenopus laevis* (type 1), *Gastrophryne carolinensis* (type 2) and *Ascaphus trueti* (type 3), two layers of epithelial tissue separated by mesenchyme were always present. The external layer corresponds to the skin of the ventral fin (stratified epithelium), the internal layer to the inner wall of the vent tube (simple cuboidal epithelium). The vent tube grows in centrifugal direction from the body wall backwards, and develops its definitive configuration.

## DISCUSSION

From the results of this study, the term vent tube deserves to be analysed. The posterior opening of the gut in anuran larvae was given several names, such as anus (HUETTNER, 1948), anal tube (INGER, 1985; CAMPBELL & CLARKE, 1998), anal opening (CEI, 1980), cloacal tube (ECHEVERRÍA & FIORITO DE LOPEZ, 1981), vent tube (ALHIG & JOHNSTON, 1986) or proctodeal tube (LAVILLA & LANGONE, 1991). The external tube is a structure formed from the epidermis as the larva grows. It is connected to the cloaca, and it could be considered a part of the cloaca of the embryo, specifically of the embryo proctodeum, an ectodermic structure. Vent tube can be interpreted, in two ways: (1) as a prolongation of the proctodeum at the expense of the ventral body skin, forming a tube from early stage 23 to the final 25, and (2) as an external tube formed just from the posterior edge of the body wall, specifically from the ventral and lateral epidermis that forms the ventral fin too. This second view is based on the tissues from which the vent tube has started its development.

However, the external tube involves two kinds of epidermal tissues in the definite vent tube: original proctodeal material (parts of the inner wall) and epithelial tissues grown a posteriori (in inner and outer walls). MCDIARMID & ALHIG (1999) assumed the vent tube to be a prolongation of the abdominal skin. This could be a wider interpretation enclosed in the second case. The inner wall of the vent tube rises from the epithelial tissue attached to the margin of the distal cloacal area. It is surrounded by the skin (epidermis and dermis) that forms the outer wall. The connective tissue grows in width, and becomes more evident between both epithelia (fig. 7). On the basis of its anatomy, perhaps better names for the tube projected from the body wall for excreting feces could be at first sight proctodeal tube, cloacal tube or vent tube. Proctodeal tube is restrictive only to a part of the cloaca, and it should not be considered as a prolongation of the original proctodeum because it has a different tissue composition. The term cloacal tube makes references to the functions and could be used in opposition to the functional connotation that anal tube has. The term vent tube is probably the best, it is a general term with no developmental connotation, unique to tadpoles, that could be better used by English speakers.

The vent tube is functional by stage 25. Its inner wall is partially covered by ciliated and secretory cells, that may probably contribute to the joining of feces into a string of intestinal

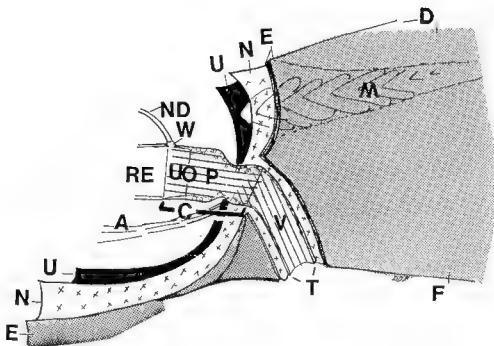


Fig 7 Schematic representation of a dissected cloaca (C) and vent tube (V) of a tadpole A. *rectus abdominis* muscle, C. cloaca, D. dorsal fin, E. epidermis, F. ventral fin, M. tail muscles, N. loose connective (mesenchyme), ND. nephric duct, P. proctodeum; RE. hind intestine or rectum, T. vent tube exit; U. peritoneum; UO. urodaeum; V. vent tube; W. nephric duct orifice

particles before they are excreted. No traces of muscles are found around the wall of the vent tube. The *rectus abdominis* muscle that is attached to the posterior wall of the body, next to the inner cloaca, has been studied by CARR & ALFEG (1992) in several tadpoles. These authors assume that this muscle could stabilize the abdomen and spiracular wall in suctorial tadpoles. Furthermore, contraction of this muscle could indirectly reduce the inner cloaca lumen, by compression. The compression over the lateral walls of the inner cloaca could help to remove part of the fecal string when it reaches the exterior.

Different cell specializations (brushborder and microvilli), formed after stage 24, are detected only in the intestines and are absent in the larval anatomical cloaca. Ciliated cells of the cloaca have their long cilia oriented to the posterior end of the body. They probably help to produce a current which conveys mucus and fecal particles through the cloaca, or they contribute to organize the fecal string before it leaves the vent tube.

No structural differences were found in the vent tube of bufonid tadpoles studied in this work. Intentionally, several tadpoles representing different anatomical and ecomorphological types, sensu ALFEG & JOHNSON (1989) (suctorial tadpole, *Aescaphus truci*, and suspension feeders, *Xenopus laevis*, *Gastrophryne carolinensis* and *Elachistocleis bicolor*), carrying medial

vent tubes, were examined. They showed similar structural organization based on epidermis and dermis. Probably this result suggests that vent tube configuration could have a taxonomic rather than ecomorphological significance.

## RESUMEN

El tubo cloacal de las larvas de *Bufo arenarum* y *Bufo fernandezae* deriva de la porción ectodérmica de la abertura intestinal embrionaria. Células epiteliales perduran como indicadoras del origen ectodérmico del proctodeo, permaneciendo en el interior de la cloaca durante todo el desarrollo larval. La región distal del proctodeo y de la epidermis larval contribuyen a formar el tubo cloacal definitivo. En el estadio 19 sólo está presente la abertura presuntiva del intestino; la cloaca larval se conforma en el estadio 20 o al inicio del 21. En el estadio 25, el tubo cloacal es funcional. Se examinaron con menor detalle especímenes de larvas con tubo cloacal medial de varios tipos (larvas tipo 1, 2 y 3) mostrando una organización tisular similar a los resultados obtenidos de la cloaca y del tubo cloacal de los bufónidos estudiados.

## ACKNOWLEDGMENTS

I am most grateful to the mentor of this research, Dr Ronald Altig (Mississippi State University), to Ronald Heyer and Roy McDiarmid (Smithsonian Institution), who provided the specimens of *Asiaphus truci*, to Dr Graciela Guerrero (Embryology Laboratory, Buenos Aires University), for comments, and to Mr Dante Jimenez (SEM Service of Centro de Investigaciones Técnicas de las Fuerzas Armadas), for technical assistance. Special thanks to the University of Buenos Aires Board that gave me financial support to make the trip to Mississippi State University (Expte. N° 931/1996), the place where I started this research.

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## Honduran amphibians

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James R. McCranie & Larry D. Wilson. – *The amphibians of Honduras*. Ithaca, New York, USA, *SSAR Contribution to Herpetology* 19, 2002: i-x + 1-625, 20 plates with 154 colour photos, 67 b&w figures, 33 tables and 58 maps. US \$ 60.00. Hardbound. ISBN 0-916984-57-5.

The book of James R. McCranie and Larry D. Wilson deals with the Amphibians of Honduras. This is the nineteenth item of the great series *Contribution to Herpetology* published by the Society for the Study of Amphibians and Reptiles (SSAR). The authors transmit us very methodically the result of more than 30 years of field work spent in one of the less known countries of Middle America.

After a brief introduction where are given the intention and the architecture of the book, a detailed material and methods part is given.

The first chapter of the book allows us to get a very good insight of the Honduran environment through fourteen pages (p. 13-26). Data are provided on geography (main regions, rivers, relief, etc.), climates and vegetation. The following chapter provides a brief history of amphibian studies in Honduras; the authors relate how our knowledge on the Honduran herpetofauna grew little by little thanks to the scientific trips that occurred from the nineteenth century.

The following chapter (p. 35-87) consists of dichotomous keys: keys for orders, genera and species were elaborated; they are illustrated by many figures and are provided both in English and Spanish. These keys allow to identify not only adult specimens but also tadpoles, alive or preserved.

The next chapter, titled *Systematic accounts*, is the most voluminous part of the book (p. 89-499). A presentation of the nine families of amphibians occurring in Honduras is given. Systematic references, range, content and etymology are presented for each genus name. An important amount of data is provided for each species through different sections: list of synonyms, range, diagnosis, description of adults and tadpoles, illustrations, remarks (mostly in systematics), ecological distribution and natural history comments, etymology, specimens examined, and other records. Let us note that there is no section devoted to the call descriptions. In fact, the authors explained (p. 10) that they have few data in this respect, so that they just gathered the existing data of the literature and included them in the natural history comments. Many distribution maps are given, where the localities are accurately indicated by dots.

Twenty colour plates with good photographs come just after the systematic accounts. Both the habitats (24 photographs) and the amphibians (130 photographs) are presented.

The three following pages (p. 501-503) deal with the possible or doubtful occurrence of several amphibians species in Honduras. With regards to the amphibian species occurring in the vicinities of Honduras, the authors indeed think that seven further species should occur in that country. Conversely, the occurrence of one frog species (*Hyla crepitans* Wied-Neuwied, 1824) is questioned.

Through the two following short chapters, the authors analyse the reproductive modes and guild structures. The guild structures are taken into account for both adults and tadpoles.

Then, two chapters are devoted to environment and amphibian conservation in Honduras. In the first one, the authors recall the major causes of extinction and insist on the fact that three of them are due to human overpopulation. Given a high birth rate in Honduras, like in many other countries of Middle America, the authors consider different scenarios of the environmental impact of human population for the future. In the second one, they estimate by an interesting "environmental vulnerability scores" (EVS) which amphibian species are prone to extinction. On the basis of the EVS they obtained for each amphibian species, they discuss the efficiency of the existing system of Honduran biotic reserves in protecting the amphibian fauna. A new plan of action to restrain environmental damage to these animals is proposed.

After a two pages Spanish summary, a glossary, where 176 unfamiliar words are defined, is available, which will be very useful to all amphibian non-specialists.

The next part is a gazetteer (p. 553-578): details are given for 699 localities (including misspellings and various spellings of localities found in previous works). A map (p. 554) with 80 selected localities indicated by dots is also provided.

The gazetteer is followed by a list of 658 references (p. 579-610), including as well ancient books or papers of the previous centuries, as the most recent papers dealing with molecular phylogeny.

Finally, two indices are provided, one to the scientific names (p. 611-619), and another one to the authors' names (p. 619-625).

This book is well done and contains the largest quantity of data ever gathered on Honduran amphibians so far. It is not only a simple updated systematic list of animals with a key and some well-referenced details about their biology and biogeography, it is also an important tool of investigation for the amphibian conservation in Honduras. Such a work is a model of what should be done, among other things, to tackle the animal conservation problems on a sound foundation. I strongly recommend it without any reserve to all people interested in the Middle-America herpetofauna and in animal conservation.



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- GRAF, J.-D. & POLLS PELAZ, M., 1989. - Evolutionary genetics of the *Rana esculenta* complex. In: R. M. DAWLEY & J. P. BOGART (ed.), *Evolution and ecology of unisexual vertebrates*, Albany, The New York State Museum: 289-302.
- INGER, R. F., VORIS, H. K. & VORIS, H. H., 1974. - Genetic variation and population ecology of some Southeast Asian frogs of the genera *Bufo* and *Rana*. *Biochem. Genet.*, 12: 121-145.

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Published with the support of AALRAM  
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du Muséum National d'Histoire Naturelle, Paris, France).

Directeur de la Publication: Alain DUBOIS.  
Numéro de Commission Paritaire: 64851.

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*Alytes* is printed on acid-free paper.

*Alytes* is indexed in *Biosis*, *Cambridge Scientific Abstracts*, *Current Awareness in Biological Sciences*, *Pascal*, *Referativny Zhurnal* and *The Zoological Record*.

Imprimerie F. Paillart, Abbeville, France.

Dépôt légal: 4<sup>e</sup> trimestre 2002.